INTRASPECIFIC VARIATION IN NUTRIENT EXCRETION BY FISH: EXPLORING STOICHIOMETRIC PARADIGMS WITH A MODEL SPECIES FOR TRAIT VARIATION

A DISSERTATION

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF CORNELL UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

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AUGUST 2015

INTRASPECIFIC VARIATION IN NUTRIENT EXCRETION BY FISH: EXPLORING STOICHIOMETRIC PARADIGMS WITH A MODEL SPECIES FOR TRAIT VARIATION

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Cornell University 2015

ABSTRACT

Nutrient excretion by consumers can alter ecosystems by changing the availability of limiting nutrients. Understanding controls on nutrient excretion is thus necessary to predict ecological dynamics. Ecological stoichiometry predicts nutrient excretion rates based on the principles of homeostasis and mass balance, and this theoretical framework effectively explains variation in excretion *among* fish species. It fails, however, to explain the substantial variation in excretion *within* species. This dissertation uses a model species for trait variation, the Trinidadian guppy (*Poecilia reticulata*), to explore why ecological stoichiometry has fared so poorly in explaining intraspecific variation in excretion.

I first used a lab study to assess traditional stoichiometric predictions for nutrient excretion. This study found the expected stoichiometric patterns for phosphorus excretion, but not for nitrogen excretion. A field survey supported these results, and it indicated that the presence of a predatory fish, *Crenicichla frenata*, explained variation in guppy nitrogen excretion. In ensuing lab studies, I sought to isolate the influence of predation risk on nitrogen excretion by rearing guppies with or without the chemical cues of this predatory fish. This approach highlighted that predator cues reduced guppy nitrogen excretion by nearly 40%, primarily by reducing food intake. Standardizing for food intake, however, revealed that predator

cues reduced guppy excretion more than food consumption. Guppies with predator cues thus excreted a smaller fraction of consumed nitrogen, retaining nitrogen more efficiently.

We suggest that reduced nitrogen excretion under predation risk reflects a waste-minimizing physiology similar to that caused by extended food deprivation. We show, however, that food deprivation takes up to 14 days to change guppy physiology, while predation risk induces even greater metabolic change within just 24 h. This result raises the possibility that guppies use predation risk as a signal of impending food restriction, immediately inducing metabolic changes to increase retention of nutrients for future needs.

In summary, this dissertation highlights that ecological stoichiometry can explain variation in phosphorus excretion but not nitrogen. Nitrogen excretion is better described by models incorporating causes of variation in metabolism. Predation risk, a major influence on consumer metabolism, is likely central to intraspecific variation in nitrogen excretion.

BIOGRAPHICAL SKETCH

Christopher Michael Dalton received a B.S. in Ecology and Evolutionary Biology from Yale University in 2006. At Yale University, Chris was advised by Dr. David Post and completed an undergraduate thesis on the effect of Double-crested Cormorants (*Phalacrocorax auritus*) on a declining anadromous fish, the alewife (*Alosa pseudoharengus*). Chris then worked on distribution and procurement projects for Fortune 500 companies while preparing his undergraduate thesis for publication. In 2009, Chris began his studies in Ecology and Evolutionary Biology at Cornell University under Dr. Alexander S. Flecker. In 2011, Chris was fortunate to add Dr. Nelson G. Hairston, Jr. as a co-advisor, formally acknowledging the strong role both Dr. Hairston and Dr. Flecker played in shaping Chris' intellectual development.

DEDICATION

Dedicated to my parents, John and Anne Dalton, for inspiring me to love the natural world

This dissertation was supported, guided, and inspired by my co-advisors, Dr. Alexander S. Flecker and Dr. Nelson G. Hairston, Jr., and by my committee member Dr. Amy R. McCune. Their wisdom, advice, and support contributed to each individual chapter and the direction of the research as a whole

ACKNOWLEDGEMENTS

I have been blessed throughout my life with supportive family, friends, and mentors. My parents were my first mentors and will always be the most important. They showed me how to be inquisitive, hard-working, dedicated, and caring. Beyond all of the innumerable sacrifices they made on my behalf, they showed me the meaning of unconditional love. For that, I simply could not be more grateful.

My siblings, John and Colleen (and their spouses Kelly and Paul, respectively), embraced their position as role model for their annoying younger brother. They helped me navigate through life's challenges and continue to inspire me and demonstrate the many meanings of kindness in their daily lives. I am grateful for their love and support.

My graduate studies would simply have not been possible without the seemingly endless generosity of my advisors, Dr. Alexander S. Flecker and Dr. Nelson G. Hairston, Jr. Both Dr. Flecker and Dr. Hairston are remarkable for their passionate curiosity about the natural world, pragmatic drive for uncovering answers, thoughtful perspective on the responsibilities of being a scientist, and unstinting commitment to developing the potential of others. They have shaped me into a better scientist, leader, and person. I feel impossibly lucky to have had the opportunity to learn from them. I am also grateful to have had the opportunity to learn from committee member Dr. Amy R. McCune, whose deep thinking on fishes, macroevolution, and all things biological, stretched me to challenge my assumptions and grow as a scientist.

I have been lucky to interact with the terrific faculty across multiple departments at Cornell University, including Dr. Cliff Kraft of Natural Resources, Dr. Jennifer Thaler of Entomology, and Dr. Monica Geber, Dr. Jed Sparks, Dr. Kelly Zamudio, and Dr. Harry Greene

of Ecology and Evolutionary Biology. I am grateful for their contributions to my intellectual and emotional development. Interactions with many other faculty including Dr. David Winkler, Dr. Christie Goodale, Dr. Lars Rudstam, among others, all helped me develop my knowledge and skills as a scientist. I also benefited from endless help from the generous staff in Corson Hall, including Marvin (Rich) Moore, Carol Damm, Patty Jordan, DeeDee Albertsman, LuAnne Kenjerska, Gary Oltz, Janeen Orr, and Jennifer Robinson, among many others. My laboratory work was greatly facilitated by the dedicated and generous assistance of lab managers Lindsay Schaffner, Guin Frederiksen, and Kim Sparks.

This research relies heavily on the contributions of diverse collaborators. I am grateful to Rana El-Sabaawi, Sonya Auer, Dale Honeyfield, David Reznick, Corey Handelsman, Emily Ruell, Cameron Ghalambor, Dale Broder, Andrew Jones, Karen Sullam, and Eugenia Zandonà for their work and inspiration. I am indebted to many undergraduate researchers who were responsible for doing the hard, unrewarding work, including Johanna Katz, Mauri Ren, Hywel Wong, Swati Sureka, Shafika Deria, Elizabeth Denning, Alicia Zhao, Priscila Cuñha, William Aragundi, Connor Benfield, Winnie Chu, Camille Tucker, Karen Tracy, and Madeline Rich.

I am grateful for funding from the National Science Foundation for a Predoctoral Fellowship, a Doctoral Dissertation Improvement Grant, and funding through DEB-0623632. For essential funding to support my research, I am also grateful to the Cornell Andrew W. Mellon Student Grant Program, the Cornell Branch of the Sigma Xi Society, the Kieckhefer Adirondack Fellowship Program, the American Society of Naturalists, the Cornell Biogeochemistry and Environmental Biocomplexity Program, the Cornell Graduate School, and the Department of Ecology and Evolutionary Biology summer research fund. I am grateful for

generous fellowship and other financial assistance from the Department of Ecology and Evolutionary Biology and Cornell Graduate School.

The graduate community at Cornell played a large role in my research. Foremost among this group was the Hair-Fleck Lab group, whose supportive and constructive perspective helped me build my research program. Thank you Marita Davison, Jennifer Moslemi, Rana El-Sabaawi, Krista Capps, Mike Booth, Carla Atkinson, Joe Simonis, Cayelan Carey, Sarah Collins, Lily Twining, Katie Sirianni, Keeley MacNeil, Rachel Abbott, Dan Auerbach, and Erin Larson for your ideas and support.

I was also blessed with some of the best friendships I have made in my life, including those with my cohort-mates Ana Longo and Alberto Lopez and office-mates Mike Booth, Findley Finseth, Morgan Mouchka, Erica Larson, David Cerasale, Ben Johnson, Marjorie Weber, Kate Eisen, and Collin Edwards. Your support helped me enjoy the good times and get through the bad. I am incredibly grateful to "the Suze" (A.K.A. Suzi Claflin) for engaging and challenging discussions and to Nancy Chen for unwavering support and friendship. I thank Rayna Bell for discussions that helped kick-start my graduate research. I am also grateful to David Cerasale and Mike Booth for providing valuable career advice that helped me navigate the difficult choices facing students seeking careers outside of academia.

I am grateful to those who helped me start my education. To David Post, Eric Palkovacs, and Torrance Hanley, for helping me explore research, conduct field studies, and build ideas. Thanks for being my cheerleaders all through this process. To David Clapp and Fran Garretson, for teaching me natural history and introducing me to ecology. To my loving grandmothers, Ma

and Nani, for always being interested in my research, and my entire extended family for your love and support.

I want to close these acknowledgements by describing my incredible gratitude for my friend, fiancé, and life partner, Katherine Walker. Kate's commitment to seeing the best in people and helping them, even at great costs, continually reminds me that happiness comes not from individual success but from collective progress. Kate's unwavering belief in my abilities helped me through even the hardest days of graduate school. Kate has helped me find who I am and has enabled me to find the meaning of real joy. I am so grateful, and feel so lucky, to have the opportunity to plan a life together with her.

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PREFACE

Reciprocal connections between ecology and evolution underlie many recent advances in ecological research (Schoener 2011). Eroded by decades of studies demonstrating "rapid evolution" in organisms from bacteria to Darwin's finches (Hendry and Kinnison 1999; Hairston et al. 2005), the barriers between ecology and evolution have crumbled, leading to a new focus on so-called eco-evolutionary dynamics (Post and Palkovacs 2009). This field seeks to understand how ecology and evolution interact, reciprocally, to dictate the structure and function of the biological world. Field studies showing genotypic variation in ecological interactions (Hughes et al. 2008), phylogenetic controls on community structure (Vamosi et al. 2009), and cascades of eco-evolutionary dynamics (Walsh and Post 2011) highlight that, in order to understand either ecology or evolution, one must incorporate both.

Ecology and evolution are linked in the framework of ecological stoichiometry, which explores the biological causes and consequences of organisms' multi-elemental nutritional requirements. This framework uses simplifying assumptions about the kinetics of biological reactions to predict environmental dynamics across space and time (Sterner and Elser 2002). Much as the Metabolic Theory of Ecology produced sweeping ecological predictions with a few physical rules (Brown et al. 2004), Ecological Stoichiometry garners insights at scales from molecules to the biosphere by broadly applying the concepts of stoichiometry and mass balance.

In a biological context, stoichiometry describes how biological processes are composed of chemical reactions that occur with fixed ratios (hence, stoichiometry). The rate of these reactions, and thus of biological processes, can be limited by a deficit in any single reactant. Consumers, moreover, can neither create nor destroy elements, enabling mass balance

accounting of elemental fluxes through their tissues. In this simple model, organisms forage to ingest and assimilate the ratios of elements necessary to serve as inputs to the reactions necessary to enable their life histories. Thus selection on organismal behavior and physiology may act, not to maximize assimilation and retention of any single resource (*i.e.*, energy), but to meet demand for the diverse nutritional substrates required for the reactions underlying life histories (Raubenheimer et al. 2009).

Because mass must be conserved, moreover, this framework can predict the production of wastes by organisms. Wastes will only be produced when there is a mismatch between a consumer's requirements for nutrients and the dietary supply of those nutrients (Frost et al. 2006). Nutrients obtained in excess of required ratios will be released into the environment. These released nutrients may reduce nutrient limitation at the base of the food web by increasing the supply of rate-limiting resources for primary producers (Knoll et al. 2009). Thus ecological stoichiometry uses two basic principles (stoichiometry and mass balance) to understand how nutrients shape the behavior and physiology of consumers and how nutrient supply and demand interact to determine the nutrients released by consumers as waste.

The roots of ecological stoichiometry lie in research on invertebrate herbivores and planktivores. In these systems, small-bodied invertebrates with relatively fixed tissue elemental composition consume vast quantities of plants and algae that vary in their elemental composition (Sterner 1990; Faerovig and Hessen 2003; Demott et al. 2010). The deficit of any single element in the primary producers could limit the growth rates of rapidly growing zooplankton populations (Urabe et al. 1997). Evolution by these zooplankters to acquire and retain these limiting nutrients (Seidendorf et al. 2010) may reduce release of limiting nutrients in wastes,

exacerbating the nutrient limitation that had produced the low nutrient algae (Elser and Urabe 1999). In turn, selection for altered life history or morphology can alter the nutrient demands of metabolism and growth (Elser et al. 2003; Ferräo-Filho et al. 2007; Costello and Michel 2013), changing patterns of nutrient resupply to the base of the food web with potential ecosystem consequences (Vanni 2002). This research thus offered powerful insights into how variation in the environmental supply of, and organism demand for, limiting nutrients could shape ecosystem function.

Researchers seeking to apply these concepts to another group of important aquatic consumers, fishes, first assessed patterns in dietary, tissue, and excretion nutrients among diverse assemblages of fish (Vanni et al. 2002). In such comparisons, researchers found the expected patterns based on mass conservation: fish species with higher nutrient diets excreted nutrients at higher rates, and fish species with higher nutrient tissues excreted nutrients at lower rates (McIntyre and Flecker 2010). Field studies probing more deeply into this variation within species, however, revealed that many of these patterns unraveled within any single species. Diet is a weak predictor of the excretion rates within wild fish species (McManamay et al. 2011; Vrede et al. 2011) and tissue nutrients can almost never be correlated with excretion nutrients in field surveys of any given species (Torres and Vanni 2007).

This dissertation explores why traditional paradigms of ecological stoichiometry have failed to explain intraspecific variation in fish excretion rates. It probes these questions using (1) controlled lab studies to probe specific mechanisms, (2) field surveys to contrast specific hypotheses, and (3) laboratory manipulations to assess a potentially important but unexplored mechanism driving variation in fish excretion: metabolic shifts induced by predation risk. The

overarching goal of this research is to understand the primary causes of variation in tissue and excretion nutrients in fish as well as their adaptive significance. By understanding the adaptive significance of this variation, this work strives to provide general insights into how selection and plasticity shape and constrain the nutrient phenotypes of consumers.

Chapter 1 describes a laboratory study to assess how dietary nutrient supply alters nutrients retained in tissue and nutrients excreted. In this study, we varied the phosphorus (P) content of an artificial diet to span above and below the expected point of nutrient limitation. We found that high P diets induced parallel increases in tissue and excretion nutrients. Luxury deposition of excess dietary P into skeletal tissue drove a positive relationship between tissue and excretion nutrients that countered the expected negative relationship between tissue and excretion nutrients based on mass balance. Standardizing for diet quality, however, enabled us to identify a negative relationship between tissue and excretion P among genetically distinct populations. These results suggest that expected patterns in ecological stoichiometry do occur within fish species but are overwhelmed by induced dietary influences on fish excretion.

Chapter 2 presents the results of a field survey designed to assess the relationships between diet quality, tissue nutrients, and excretion rates of one species of fish together with other ecological variables across 36 populations. This study found resource-driven influences on fish tissue nutrients. Sites with more light had more resources, and fish in sites with more resources had more lipid-C and protein-N than fish in low light sites. Tissue and excretion nutrients, however, were almost entirely decoupled, and excretion nutrients most strongly reflected the presence of a predatory fish species. We posited that behavioral change under predation risk drove the predator-related reduction in excretion rates. This set of results points to

predation risk as the central driver of intraspecific variation in excretion rates, largely independent of diet quality or tissue nutrient composition.

Chapters 3 through 5 probe the influence of predation risk on fish excretion rates and the evolutionary significance of those predator effects using laboratory manipulations.

Chapter 3 describes an experiment which exposed a species of prey fish, Trinidadian guppies (*Poecilia reticulata*), to 7 weeks of predation risk on two different quality diets. Guppies under predation risk consumed much less food and had 40% lower excretion at the termination of the experiment, a reduction driven by their lower rates of food consumption, smaller body size, and, intriguingly, an otherwise unexplained 10% residual reduction in excretion independent of fish size or food consumption. This chapter indicates that predators do in fact reduce fish nutrient excretion rates, though the strong reduction in food consumption under predation risk raises the question of whether this reduced excretion simply reflects lower food consumption under risk or some other influence on the physiology of prey.

Chapter 4 describes a food restriction experiment to probe the independent influence of predation risk and food consumption as causes of variation in nutrient excretion by guppies. Over periods from 3-14 days, the study assessed change in various guppy traits under both predation risk and food deprivation. This study found that N excretion was influenced by predation risk within three days of initiation of treatments, and N excretion reflected the presence of predation risk independent of any change in food consumption. The effect of our food restriction on every measured guppy trait was parallel to the influence of predation risk, but predation risk effects were disproportionately large only on excretion rates. Moreover, food restriction did not influence excretion rates until the conclusion of the 14 day trial, whereas

predation risk affected excretion rate on the third day of the trial. This result points to change in N excretion as an adaptive response to predator-induced food restriction that increases retention of a potentially limiting nutrient.

Chapter 5 is an investigation of the mechanism by which guppies alter their excretion under predation risk, and the physiological consequence of shifts in excretion. By measuring both the excretion and respiration rates of guppies, this study assessed whether changes in total energy budgets under predation risk are sufficient to explain predator-induced changes in guppy excretion rates. Contrary to this expectation, we found that changes in excretion rate were induced by predators independent of changes in total energy metabolism. Guppies excreted less N per unit of oxygen consumed when predators were present. Unlike energy metabolism, moreover, N excretion reflected only short-term (*i.e.*, <24 h) exposure to predation risk cues, whereas respiration rate reflected the long-term exposure to predation risk. The change in metabolic use of N, moreover, resulted in predator cue guppies slowing the loss of N due to turnover and catabolism of endogenous N stores, increasing the N efficiency of predator-cue exposed guppies and thus the demand for more N.

Taking these results together, I present evidence that diet quality can be an important determinant of the variation in tissue nutrients within fish species, but that predation risk can drive substantial and confounding variation. We explore in detail the causes, mechanisms, and consequences of plasticity in fish tissue and excretion nutrients under predation risk. We link potential adaptive explanations for changes in excretion (conservation of limited N under food restriction) to this trait, enabling us to predict that the responses of guppy excretion to predation risk may be general among fish and not specific to this system. By understanding the potential

evolutionary significance of induced changes in the nutrient recycling traits of consumers, we are able to draw potentially general conclusions about how selection acts to alter suites of organism traits under certain environmental conditions.

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CHAPTER ONE: THE ROLE OF DIET AND GENETIC DIFFERENTIATION IN PATTERNS OF INTRASPECIFIC VARIATION IN THE NUTRIENT STOICHIOMETRY OF FISH

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ABSTRACT

Fish are important in nutrient cycles due to their excretion and sequestration of potentially limiting nutrients. Mass balance models predict fish diet and tissue nutrient content will largely control excretion by varying the consumer's supply and demand for nutrients. Studies of intraspecific variation in excretion in fish, however, often fail to find such patterns. Here, we explore the role of diet, tissue nutrient content, and genetic differentiation among fish populations as causes of intraspecific variation in nutrient excretion in the Trinidadian guppy (*Poecilia reticulata*). We reared guppies from four populations on diets with high and low phosphorus (P) content, and we measured their nitrogen (N) and P excretion, as well as their tissue carbon (C), N and P content after prolonged dietary treatment. We assessed the contribution of diet P and population to the tissue nutrients of guppies, and the relationship between tissue and excretion nutrients. Diet quality induced parallel increases in tissue P and P excretion. Analysis of our individual fish excretion rates found limited evidence for the predicted negative relationship between tissue and excretion nutrients. Analysis of population-level averages, after correcting for the positive effect of diet on tissue and excretion P, suggests a negative correlation between tissue and excretion P caused by divergence among populations. No such pattern was indicated for N excretion. Guppies from environments with varying predation risk differed in some stoichiometric traits, especially excretion nutrients. We suggest that genetic divergence in behavioral traits may underlie these differences in our lab reared fish.

INTRODUCTION

Sequestration and excretion of nutrients by consumers can exacerbate or offset the effects of nutrient limitation at the base of the food web (Hood et al. 2005; Small et al. 2011; Layman et al. 2013). Understanding the constraints on nutrient release by consumers is thus necessary to understand ecosystem dynamics (Elser and Urabe 1999). The nutrients released and sequestered by consumers are constrained by the principle of mass balance; every atom of nutrient ingested by a consumer must either be retained in its tissue or released in its waste (Sterner and Elser 2002). By this framework, the rate of waste release by a consumer can theoretically be predicted just by knowing the amount of nutrient in its food and the amount of nutrient required for its growth.

This framework leads to two predictions for patterns of variation in excretion rate. A consumer's excretion should scale positively with the amount of nutrient in its food and negatively with the amount of nutrient in its tissue. Both of these predictions have been empirically supported in studies of zooplankton and interspecific variation in fishes (Elser and Urabe 1999; McIntyre and Flecker 2010). Results from studies of any single species of fish, however, are less clear. While some studies find positive relationships between excretion and food nutrient composition (Higgins et al. 2006; Torres and Vanni 2007), others find no pattern (McManamay et al. 2011; Villéger et al. 2012). No studies recover an influence of tissue nutrient content on excretion rate (Torres and Vanni 2007). Researchers must thus consider why this framework has produced such inconsistent results. Are these mechanisms not operating within fish species or are other mechanisms obscuring their influence?

Patterns of intraspecific variation in excretion could be obscured by plasticity in fish tissue nutrient demands, or flexible homeostasis. The aforementioned simple model for nutrient wastes assumes strict homeostasis, wherein consumer tissue nutrient content is inflexible and rigorously constrained by the evolution of its body plan (Persson et al. 2010). By this model, a consumer must release any nutrient in excess of its fixed structural requirements, causing a positive relationship between diet nutrient content and excretion rate. If, however, consumers can store excess dietary nutrients in their tissues (analogous to luxury consumption in algae; (White et al. 2006)), they could sequester dietary excesses instead of excreting them, decoupling dietary and excretion nutrients. Moreover, if dietary nutrient supply exceeds this luxury deposition, then tissue nutrients and excretion nutrients will both increase with dietary nutrient content. This positive correlation would reverse the expected negative relationship between tissue and excretion nutrients.

Field studies have reached divergent conclusions regarding just how fixed the nutrient demands of fish growth really are (Sterner and George 2000; Hendrixson et al. 2007; Vrede et al. 2011), and thus how likely flexible homeostasis is to interrupt the expected relationship between tissue and excretion nutrients. Indeed, field studies are handcuffed by the difficulty of characterizing the diet quality of wild fish, as well as potential non-linear threshold responses (Bureau and Cho 1999). Here we seek to assess how diet quality affects tissue and excretion nutrient content using a laboratory study on a model system for intraspecific variation in excretion and tissue nutrients, the Trinidadian guppy (*Poecilia reticulata*).

Guppies have long been studied for the rapid evolution of their life history traits under release from predation (Reznick and Endler 1982), and more recent studies have revealed

extensive variation in their tissue and excretion nutrients (Kohler et al. 2012; El-Sabaawi et al. 2012b). Guppies occur ancestrally in high predation (HPred) environments, where predators impose high extrinsic mortality on guppies, but these HPred guppies have abundant nutrient-rich invertebrate foods (Zandonà et al. 2011; El-Sabaawi et al. 2012b), potentially due to a trophic cascade. Across the northern range mountains of Trinidad, guppies have repeatedly invaded upstream reaches where predators do not occur, and, in these low predation (LPred) environments, guppies rapidly evolve altered life history traits (Reznick et al. 1996) while increasingly foraging on nutrient-poor detrital foods (Zandonà et al. 2011; El-Sabaawi et al. 2012b).

Previous studies suggest that this divergence in diet quality may influence guppy excretion rates. HPred guppies, with more nutrient-rich invertebrate diets, excrete more nutrients (nitrogen (N) and phosphorus (P)) than LPred (Bassar et al. 2010; Kohler et al. 2012); however, many other HPred-LPred trait differences could also explain these intraspecific differences (Reznick and Bryga 1987; O'Steen et al. 2002; Handelsman et al. 2013). Guppies do have high intraspecific variation in tissue nutrients (El-Sabaawi et al. 2012b), making them a candidate for studying the role of diet quality as a cause of variation in both excretion and tissue nutrients. The parallel genetic divergence among guppy populations along a gradient from high to low predation, moreover, provides an opportunity to compare such diet-induced variation to that caused by genetic differentiation under divergent environments.

In this study, we assess the hypothesis that diet can affect both tissue and excretion nutrients using a dietary P manipulation, using guppies from four populations (two separate origins of an HPred-LPred contrast). We assess the hypothesis that tissue and excretion nutrients

are negatively correlated by comparing tissue and excretion nutrients within and among population and diet treatments. Finally, we assess the influence of diet quality and genetic divergence along a parallel gradient in predation risk on the tissue and excretion nutrients of Trinidadian guppies.

METHODS

Overview

Guppies from one HPred and one LPred site in each of two rivers were bred in common garden lab conditions. Guppies from each of these four populations were reared on diets that were either replete with or deficient in P content. After a ten-week growth period, guppies were assayed for excretion rate and for tissue C, N and P. Effects of ancestral predation environment (HPred vs. LPred) and diet P content (high vs. low) on excretion and tissue stoichiometry were assessed using a model-comparative framework.

Experimental design

Guppies were collected from the Guanapo and Aripo drainages of the Northern Range Mountains of Trinidad. Within each of the drainages, we collected fish from a high-predation locality where guppies coexist with a diversity of large piscivorous fish, and a low predation locality that lacks large piscivorous fish. HPred and LPred guppy populations within each of these two drainages have previously been shown to have diverged genetically in life history traits (Reznick 1982; Torres-Dowdall et al. 2012). Wild-caught females from each population were kept individually either in 20 L glass tanks without recirculating water (Guanapo fish) or in 3 L tanks in recirculating systems (Aripo fish) (12L:12D, temperature 25 ± 1°C) and fed twice daily (AM: Tetramin^{TM} tropical fish flake paste, PM: hatched immature *Artemia* spp.). Second

generation lab-born guppies from the Aripo and first generation lab-born guppies from the Guanapo were used in the experiment.

Our experimental design emphasizes assessment of diet P content and HPred-LPred effects within rivers. Our design enables these comparisons, but unexpected logistical difficulties constrained laboratory breeding operations, with three impacts on the design of this experiment: (1) they limited the sample size of Aripo guppies available for experimentation; (2) they necessitated using 1st lab generation, and not 2nd lab generation guppies in the Guanapo; (3) they required different rearing conditions for the Guanapo and the Aripo. These impacts limit our insight from this experiment in three ways: (1) smaller sample sizes reduce the power to detect interaction effects between treatments and populations; (2) use of 1st lab generation guppies in the Guanapo reduces confidence that HPred and LPred differences in the Guanapo are not impacted by maternal effects; (3) differences between the Guanapo and Aripo Rivers cannot be attributed to genetic differentiation among rivers, as the two rivers were subject to different rearing designs and were at different ages and sizes at the time of the experiment. Despite these limitations, our design enables rigorous assessment of Diet Quality and HPred-LPred effects within each river, as described below.

A total of 14 female guppies from Aripo HPred and 14 from Aripo LPred, all between 24-35 days old, were randomly assigned to one of two treatment diets (n = 7 per predation history × diet treatment). 18 female guppies from Guanapo HPred and 18 from Guanapo LP, all of reproductive age and from first lab generation stocks, were randomly assigned to two treatment diets (n = 9 per predation history × diet treatment). Aripo guppies were stocked into 1.5 L tanks on a recirculating zebrafish system (Aquatic Habitats, Apopka, FL) with collective

filtration. Guanapo guppies were randomly assigned to treatments and housed in groups of three in stand-alone 20 L tanks filled with 19 L of water with physical and carbon filtration. Tanks were randomly assigned to locations across shelving units. Water for all guppies was provided from a central source tank that contained deionized water, Instant Ocean ® and sodium bicarbonate (Instant Ocean ®: 0.88 g L^{-1}; Sodium Bicarbonate: 0.14 g L^{-1}).

For 14 weeks (Aripo) and 11 weeks (Guanapo), guppies were fed one of two standardized treatment rations twice daily. Treatment diets consisted of either a low P or a high P content that were designed following diet specifications in Shim and Ho ((Shim and Ho 1989); Supporting Information Table 1). The high P diet had percent P comparable to a high quality invertebrate diet (0.81% of dry weight in P), while the low P diet was expected to limit guppy growth rate (0.26% P; Shim & Ho 1989). Other diet constituents were held constant among treatments. The quantities of diet fed were aligned to be isocaloric with those in the protocols of previous researchers (Reznick 1983) and never were lower than 7% of body weight per day. Uneaten food was rarely noted in any tank and, when present, was removed from each tank at the end of the day using a plastic pipette.

Response variables

The post-feeding excretion rate of each fish was measured on the last day of the experiment. Within 60 minutes of feeding, guppies were removed from their experimental tanks, introduced into plastic beakers containing 250 mL of filtered tank water (GF/F with pore size = 0.7 μm, Whatman), and placed in an opaque shelter to minimize disturbance during the incubation. After 20 minutes, we collected subsamples from each beaker using a 60 mL plastic syringe. We collected a second sample from each beaker after another 60 minutes. Prior trials

revealed that 20 minutes of acclimation was sufficient to alleviate handling stress, and 60 minute incubations minimized fasting effects (Whiles et al. 2009). Subsamples for soluble reactive phosphorus (SRP) and ammonium (NH_4^+) analysis were filtered through ashed filters (GF/F Whatman, pore size = 0.7 μm), refrigerated within 20 minutes of collection, and analyzed within 12 hours. NH_4^+ concentrations were measured on an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA), equipped with a UV filter (Holmes et al. 1999; Taylor et al. 2007), with a method detection limit of 0.2 μg N L^{-1}. SRP was determined using the molybdate method (Murphy and Riley 1962; Stainton et al. 1977). Samples were measured on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA), with a method detection limit of 1.25 μg L^{-1}, though analysis of standard samples repeatedly detected P at 0.5 μg L^{-1}. Hourly excretion was estimated as the difference in nutrient concentration between the two samples divided by the length of the incubation, in hours, after correcting for the volume of water in the beaker during the incubation. Controls used in an initial pilot study never exceeded 5% of fish excretions and were not performed for laboratory measurements.

At the conclusion of the excretion measurement, guppies were measured for length and weight, sacrificed using MS-222, placed on ice, and frozen to -10°C within 1 hour. For body nutrient analysis, guppy digestive tracts were dissected and discarded, and the combined somatic and reproductive tissues of each guppy were dried to constant mass at 55°C. Dried guppies were then weighed and ground into a homogeneous powder using a Wig-L Bug® tissue grinder. Subsamples (2 mg for C and N, 20-40 mg for P) of guppy tissue were then assayed for C, N and P content following methods described in El-Sabaawi et al (2012b). Briefly, 2 mg subsamples of homogenized tissue were weighed to the nearest 0.001 mg, and the percent C and N of each sample was assayed (Vario EL III elemental analyzer, Elementar, Hanau Germany). The

remainder of the guppy tissue was weighed, ashed in pyrex vials at 500°C for 2 h and digested in 1N HCl at 105°C to facilitate dissolution of P. SRP of the resulting solution was then quantified using serial dilutions and the molybdate blue method. Spinach and bone meal standards NIST SRM 1486 and SRM 1570a were used as known reference points in the analysis to ensure complete digestion and accurate estimates of tissue P content.

Statistical analysis

Body stoichiometry and excretion data were analyzed in R (R-Team 2010) using linear mixed models. The influences of diet P content and ancestral predation environment (HPred vs. LPred) on response variables were the main focus of our analysis and were included as fixed effects in models. River of origin (Guanapo vs. Aripo) was also included as a fixed effect in the analysis but is confounded with the age of the fish, lab generation number, and rearing environment and cannot isolate genetic differences among rivers. The non-independence of Guanapo fish reared in the same tank was accounted for by including "tank" as a random effect in every model.

The most complex, biologically-feasible models were simplified to the best fit models using likelihood-ratio tests and Akaike Information Criteria (AIC) score comparison (Akaike 1974). N excretion rates, which scale allometrically with weight, were corrected for the expected ¾ power scaling of metabolism with size by dividing measured excretion by fish weight raised to the ¾ power (Brown et al. 2004; Torres and Vanni 2007). P excretion (log-transformed) did not increase with size, which is not uncommon in intraspecific datasets (Torres and Vanni 2007), and was not size-corrected, but rather modeled per fish. Suitability of data variance distributions to these analytic methods was validated using the R package *Global*

Validation of Linear Model Assumptions (Pena and Slate 2006) on comparable models without random effects and by visual inspection of plots of variance distributions and residuals.

RESULTS

Dietary effects on tissue and excretion nutrients

Guppy tissue P decreased slightly but significantly on the low P diet, suggesting flexible homeostasis (Fig.1A; Supplemental Information Table 4; likelihood ratio test (LRT): df = 1, χ^2 = 4.02, p = 0.045; diet effect = 0.15% ± S.E. = 0.07%). The effect of diet on tissue P is also reflected in the best models for tissue C:P and N:P (Supplemental Information Tables 6-7; Supplemental Information Fig. 1E-F). P excretion increased strongly with dietary P content as predicted by the mass balance model (Fig.1B; Supplemental Information Table 9; LRT: df = 1, χ^2 = 23.5, p < 0.001; diet effect (log-transformed) = 1.25 ± S.E. = 0.22).

Neither tissue N nor N excretion was affected by diet nutrient content (Supplemental Information Fig. 1B & Fig. 2A; Supplemental Information Table 3 & Table 8), but tissue C marginally increased on the low P diet (LRT: df = 1, χ^2 = 2.95, p = 0.09, Supplemental Information Fig. 1; Supplemental Table 2). This response is consistent with aquaculture studies finding increased lipid (and thus C) deposition under dietary P restriction (Skonberg et al. 1997).

Relationships between tissue and excreted nutrients

We first analyzed global relationships between tissue and excreted nutrients. Across all populations and diet treatments, guppy P excretion was not related to tissue %P (P: LRT: df = 1, χ^2 = 0.25, p = 0.62). Within each diet treatment, tissue and excretion P were weakly negatively related (LRT: df = 1, χ^2 =2.98, p = 0.08; slope = -0.78 ± S.E. = 0.39 μg P hr^{-1} per each percent

P), though this relationship was caused by differences among the populations from the two rivers (LRT, removing Tissue %P from model for P excretion with a river effect: df = 1, $\chi^2 = 0.51$, p = 0.48). Because the guppies from the two rivers were of different size classes and were reared in different conditions, it is impossible to assess if this "river" effect is an artifact of this size difference, a product of the different conditions, or a genetic difference in tissue and excretion nutrients among populations.

We also analyzed patterns in population mean tissue and excretion P (Table 1; Fig. 2). Among the eight population × treatment combinations, we assessed models for population-mean, log-transformed P excretion with all combinations of one and two of the following effects: diet P treatment, ancestral predation environment, river of origin, and population mean tissue %P (models with three terms are over-parameterized for n=8 and never had ΔAICc < 10). The best model from among the ten candidate models included effects of diet treatment and tissue P content (Fig. 2; Table 1; low P diet effect: t = -4.72, p = 0.005; tissue %P effect: t = -3.89, p = 0.012). This model explained 85% of variation in P excretion among populations, while tissue %P or diet treatment alone explained 20% and 41% of variation in P excretion, respectively. While these relationships are strong, the small number of replicates of population × treatment combinations means that a model with no fixed effects also received substantial support (Table 1).

Across all populations, individual-level N excretion was not related to tissue N content (df = 1, $\chi^2 = 0.35$, p = 0.56). Including diet or population effects did not alter this result. In all tests, models for excretion N without tissue %N were better supported than models with this

tissue N effect (Tissue N effect as estimated by LRT: models with diet: df = 1, $\chi^2 = 0.23$, p 0.63; models with river: df = 1, $\chi^2 = 0.35$, p = 0.56).

We repeated the analysis of population mean tissue and excretion nutrients for N (Table 2). As was the case for models of individual variation in tissue and excretion N, there was no relationship between tissue and excretion N. Instead, the best model only had an effect of ancestral predation environment. Populations from LPred sites had lower N excretion than populations from HPred sites (t = -2.82, p = 0.03).

Principal Components Analysis of Stoichiometric Phenotype

Summary of individual components of the guppy stoichiometric phenotype (*i.e.*, Tissue %C, %N, and %P, N and P excretion) can be found in Supplemental Information (Supplemental Information Fig.1-2; Supplemental Information Tables 2-9). Because many of these measures are confounded, here we present the results of a principal components analysis to assess patterns of variation and covariation in these measures and to assess the contribution of treatment effects to these reduced axes of variation.

Principal component analysis decomposed 84% of the variation in tissue and nutrient stoichiometry onto three axes of variation (Table 3; Fig. 3). The first axis (PC1) predominately reflected tissue nutrients, with excretion accounting for only 27% of the total loadings onto PC1, which was generated primarily by positive loading of tissue %C and negative loadings of tissue %N and %P. N and P excretion dominated loadings onto the second axis of variation (PC2), accounting for more than 3× as much variation as the three tissue nutrient measures combined (Table 3). Both N and P excretion loaded positively onto this second axis of variation. A third

axis of variation (PC3) also accounted for an incremental 16% of the variation in this dataset and was generated by strong negative loadings from tissue %P and P excretion and positive loadings from N excretion.

Variation in PC1 scores was best explained by differences between the two rivers (Aripo vs. Guanapo; Fig. 3B & 3C), and no other model received strong support (Fig.4A; Supplemental Information Table 10). Fish from the Guanapo River had higher PC1 scores (estimate = 2.23 ± S.E. = 0.29), and thus higher tissue %C and lower tissue %N and %P than fish from the Aripo River. As noted above, because of the multiple confounding variables related to the age, size, and rearing conditions of fish from the two different rivers, it is impossible to discern whether this reflects a true "river of origin" effect or a byproduct of other differences between fish from the two rivers.

Analysis of individual components of tissue nutrient composition (*i.e.*, %C, %N, %P; Supplemental Information Tables 2-4) support a general lack of strong treatment or ancestral predation environment effects on tissue nutrients, except for a marginal relationship between ancestral predation environment and tissue N (Supplemental Information Fig.1; Supplemental Table 3; LRT for predation effect: df = 1, $\chi^2 = 3.56$, p = 0.06). Additionally, we previously established that diet is related to tissue P. This diet effect on tissue P, however, is captured in PC3 (see below).

PC2 was best explained by the guppy's ancestral predation environment and diet quality (Fig.3B; Supplemental Information Table 11). Guppies on the low P diet and from LPred environments had lower PC2 scores (diet effect: estimate = -0.60 ± S.E. = 0.29; predation effect: estimate = -0.82 ± S.E. = 0.29). A model with a predation environment × diet quality interaction

also received substantial support, suggesting that the effects of the low P diet were reduced in guppies from LPred environments, though this effect is apparent only in the Aripo guppies (Fig.4B).

Lower PC2 scores in LPred guppies suggests lower N and P excretion, and the best models for N and P excretion include an effect of ancestral predation environment (Supplemental Information Fig.2; Supplemental Information Table 8-9). Similarly, lower PC2 scores for low P diet guppies suggest lower excretion rates, though this is only true for P excretion (Supplemental Information Fig.2; Supplemental Information Table 9) and not N excretion (Supplemental Information Table 8).

PC3 was best explained by diet alone (Fig.4C; Supplemental Information Table 12). Guppies on the low P diet had higher PC3 scores (diet effect estimate = 0.93 ± S.E. = 0.20), reflecting lower P excretion, tissue P, and higher N excretion. No other model for PC3 received substantial support. Repeating this analysis for each river separately yielded comparable results.

DISCUSSION

We used a controlled laboratory manipulation of diet nutrient content to test for three potential mechanisms of variation in nutrient excretion: dietary nutrient content, tissue nutrient content, and genetic population divergence. We find evidence that diet quality and intraspecific genetic divergence influence excretion rates, and that body nutrient composition alters P excretion rates *among* populations. We consider these results in the context of field surveys for patterns in nutrient excretion, mechanisms controlling excretion rate in fish, and the challenges of analyzing stoichiometric datasets.

Homeostasis and plasticity in tissue and excretion nutrients

Mass balance models predict that nutrient excretion should be positively related to the supply of dietary nutrients, but negatively related to tissue nutrient content. Guppy P excretion was positively related to P supply, as low P diet guppies had lower P excretion than guppies fed high P diets (Fig.1A). This result is consistent with a recent meta-analysis correlating diet and excretion stoichiometry in diverse fishes in aquaculture contexts (Moody et al. 2014), suggesting this effect may be general among fish with variable quality diets.

Contrary to expectations, however, individual variation in P excretion was unrelated to tissue P content. This lack of correlation may reflect the confounding influence of diet quality and genetic divergence on tissue and excretion P. Diet quality induces parallel patterns in tissue and excretion P. Fish with high P diets had higher tissue P content and higher P excretion (Fig.1). Principal components analysis supports this assessment, as both tissue and excretion nutrients load negatively onto PC3, and the low P diet is associated with higher PC3 scores (Table 3; Fig. 4C). These results are concordant with recent meta analyses of aquaculture studies, which find positive relationships between diet P content and both fish tissue and excretion P (Benstead et al. 2014; Moody et al. 2014).

By accounting for the parallel influence of diet on tissue and excretion nutrients, however, we present evidence for a negative correlation between tissue %P and excretion P related to differences among populations. This negative correlation is suggested by the opposite direction of the loading of tissue %P and P excretion onto PC1 (Fig.3; Table 3) and further indicated by analysis of population means (Fig. 2; Table 1). Analyzing patterns in population mean stoichiometry is comparable to studies assessing interspecific comparisons, which often regress species means or measures of only a handful of individuals from each species (Vanni et

al. 2002; McIntyre and Flecker 2010). We found that such an analysis of our P excretion rates could explain 85% of the variation among treatment × population interactions with only two terms: tissue %P content and diet P. Though a model with *no* fixed effects is nearly as well-supported, these data do suggest that differences in tissue and excretion P among genetically diverged populations may follow standard paradigms of mass balance models, but only if diet quality can be accurately assessed.

Our results thus support the notion that flexible homeostasis in fish obscures potential negative relationships between tissue and excretion P. Flexible homeostasis has been noted in some field studies of fish tissue stoichiometry but not others (Sterner and George 2000; McManamay et al. 2011; Vrede et al. 2011; El-Sabaawi et al. 2012a). Mechanistic studies demonstrating "luxury deposition" of excess dietary C into lipid stores (Henderson and Sargent 1981; Arzel et al. 1994) and excess dietary P into skeletal stores (Lall and Lewis-McCrea 2007) indicate that such flexible homeostasis is likely universal in fish (Benstead et al. 2014), and can likely be assumed even if it cannot be detected. Notably, the degree of flexible homeostasis for P in fish likely exceeds that of invertebrate organisms, because fish can greatly vary the storage of P in skeletal tissues (Lall and Lewis-McCrea 2007).

Our evidence, moreover, points to this flexible homeostasis as a factor that can obscure the negative relationship between tissue and excretion nutrients among genetically differentiated populations (Fig. 2). Specifically, individuals *within* a genetically-homogeneous population will likely show a positive relationship between tissue and excretion P caused by the parallel plastic effects of diet quantity and quality (*e.g.*, Fig. 1). Genetic differences *among* populations in the P requirements of growth, underlain by genetic divergence in body plan, may nonetheless generate

a negative relationship between tissue and excretion nutrients that can only be assessed after diet quality is factored into the analysis. Because it can be difficult to assess diet quality over the multi-week timescales that influence fish physiology (Auer et al. 2012), detection of negative relationships between tissue and excretion nutrients may continue to falter even if this mechanism occurs widely among fish.

Notably, this negative relationship between tissue and excretion nutrients was not noted in any analysis of patterns of N excretion among or within populations. This lack of relationship between tissue and excretion N may stem from N's important role in energy metabolism. Fish intensively use food proteins for energy (DeSilva and Anderson 1995), and most N excretion in fish results from release of toxic ammonium as a byproduct of catabolism of amino acids for energy-yielding reactions (Mommsen and Walsh 1992; Kajimura 2004). P excretion in fish, on the other hand, is under homeostatic control and largely occurs via urinary pathways (Rodehutscord et al. 2000). Thus, N excretion is much more likely to reflect processes related to energy metabolism, independent of tissue nutrients, whereas P excretion will reflect the balance of tissue composition related to tissue growth and maintenance.

Genetic divergence of tissue nutrient content and excretion along a replicated ecological gradient

Similar to field surveys (Pilati and Vanni 2007; Vrede et al. 2011; El-Sabaawi et al. 2012a), we found significant variation in tissue and excretion nutrients of fish populations from different environments (Figs 1 and 4). Unlike field studies, however, we used common garden breeding and genetically-diverged populations (Willing et al. 2010) to attribute this variation to local genetic differentiation along a replicated ecological gradient. Guppies from HPred sites

tended to have higher excretion rates than guppies from LPred sites (for N and P; Figure 4B; Supplemental Figure 2; Supplemental Tables 8-9). This mirrors measures made from mesocosm studies (El-Sabaawi et al. 2015), and the persistence of these differences after 1-2 generations in the lab suggests genetic divergence along a replicated predation gradient may contribute to HPred and LPred divergence in excretion rates.

We can only speculate as to the potential adaptive significance of this difference in excretion rate. HPred guppies do tend to have higher tissue N and lower tissue P than LPred, perhaps altering the availability of nutrients to be excreted (Supplemental Figure 1). Additionally, as in mesocosm studies (Bassar et al. 2010), our behavioral observations (only of Aripo guppies) suggest that HPred guppies feed more intensely on high quality food items than LPred. During five-minute feeding trials in the lab, LPred made significantly fewer pecks at provided food than did HPred ($t = -2.944$, $p = 0.001$) (Supplemental Figure 3). Since feeding is one of the primary influences on excretion rate in fish (Lovell 1998), it is possible that this increased feeding rate on high nutrient foods caused the higher excretion of HPred guppies. This would provide an intriguing link between selection for guppy behavioral traits and rates of nutrient processing, but any definitive assessment of such patterns requires further investigation.

Working with stoichiometric datasets

As can be seen in our principal components analysis, measures of fish tissue nutrients (and excretion nutrients) can be highly confounded. Tissue nutrients are especially prone to such issues when expressed as nutrient ratios or as percent of dry weight, because changes in nutrient ratios or percent of dry weight can have two sources: the numerator and denominator. In this

case, it does not make sense for us to separately assess the independent changes of tissue C, N and P, as all basically fall along a single axis of variation (Fig. 3). We suspect this axis of variation is caused primarily by variation in a fish's lipid content, which can increase fish dry weight and decrease the percent of that dry weight composed of other elements (Hartman and Brandt 1995; Sullam et al. 2014). The decreases in other elements does not reflect any reduction in the allocation of the consumer to those stocks of nutrients, but rather a byproduct of increases in allocation to other nutrient stocks. We recommend using methods to either account for covariation among such measures, as we use here, or using allometric perspectives of total tissue elemental pool size (as in (Dalton and Flecker 2014; Sullam et al. 2014)).

ACKNOWLEDGEMENTS:

We thank Amy McCune for use of laboratory facilities and Amy McCune, Nelson G. Hairston, Jr., and the HairFleck Lab Group for comments that substantially improved this manuscript. We gratefully acknowledge Rich Moore, Amanda Cass, Ted Hermann, Mauri Ren, Hywel Wong, and Johanna Katz for assistance with laboratory rearing and feeding of guppies, and Corey Handelsman and Emily Ruell for training and guidance on rearing and feeding guppies. Funding for this research was provided by an NSF Frontiers in Integrative Biological Research Grant (DEB 0623632), the Cornell University Program in Biogeochemistry and Environmental Biocomplexity, and the Cornell University Andrew W. Mellon Foundation Grant. Chris Dalton was supported by an NSF Predoctoral Fellowship. Research was conducted under Cornell IACUC Protocol 2008 – 0106.

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TABLES

Table 1: Models for treatment, population, and *population average* tissue %P effects on *population average* P excretion (log-transformed). Models with both tissue %P and diet or no fixed effects received the strongest support, suggesting there may be a negative relationship between tissue and excretion P, after accounting for the influence of diet. 'Pred' represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and 'Diet' represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). 'River' refers to the river of origin of each guppy (Aripo or Guanapo). Models with more than 2 terms were over parameterized and never received support (*i.e.* ΔAICc >10). The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Population Average P Excretion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Population Average %P + Diet	28.1	0.0	1.00	0.33	1.0
No Fixed Effects	28.5	0.4	0.83	0.27	1.2
Diet	29.9	1.8	0.41	0.13	2.5
River	30.3	2.2	0.34	0.11	2.9
River + Diet	31.1	2.9	0.23	0.08	4.3
Population Average %P	32.4	4.2	0.12	0.04	8.3
Pred	32.9	4.8	0.09	0.03	10.8
Pred + Diet	37.1	9.0	0.01	0.00	88.1
River + Pred	37.5	9.4	0.01	0.00	110.3
River + Population Average %P	37.8	9.7	0.01	0.00	124.8
Population Average %P + Pred	40.7	12.5	0.00	0.00	530.8

Table 2: Models for treatment, population, and *population average* tissue %N effects on *population average* N excretion (size corrected). A model with only the ancestral predation environment of the guppy received the most support, suggesting genetic divergence in excretion rate along a replicated ecological gradient. 'Pred' represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and 'Diet' represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). 'River' refers to the river of origin of each guppy (Aripo or Guanapo). Models with more than 2 terms were over parameterized and never received support (*i.e.* ΔAICc >10). The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum wi for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for N Excretion (Size corrected)	**AICc**	**ΔAICc**	**Rel. lik.**	**w_i**	**w_i ratio**
Pred	59.0	0.0	1.00	0.59	1.0
No Fixed Effects	60.2	1.2	0.56	0.33	1.8
%N	65.6	6.6	0.04	0.02	26.4
Diet	65.7	6.7	0.04	0.02	28.5
River	65.8	6.7	0.03	0.02	28.8
%N + Pred	68.2	9.2	0.01	0.01	98.2
Pred + Diet	68.2	9.2	0.01	0.01	99.7
River + Pred	68.3	9.3	0.01	0.01	102.3
River + %N	71.7	12.7	0.00	0.00	570.5
%N + Diet	74.9	15.8	0.00	0.00	2711.7
River + Diet	75.0	16.0	0.00	0.00	2978.6

Table 3: Summary of first three principal components from PC analysis of three measures of tissue nutrients and two measures of excreted nutrients. PC1, PC2 and PC3 comprise the majority of variation in tissue and excretion stoichiometry among 58 guppies from four populations fed two diets. PC1 is generated primarily from %C, %N, and %P. PC2 is generated primarily from N and P excretion. PC3 reflects both excretion rates and tissue %P.

	PC1	PC2	PC3
Percent of variance	47.2	22.3	15.5
Cumulative percent of variance	47.2	69.5	85.0
Tissue %C loading	0.57	-0.18	0.07
Tissue %N loading	-0.56	0.13	0.15
Tissue %P loading	-0.48	0.12	-0.53
Size Corrected Excretion N loading	0.08	0.84	0.46
Log-transformed P Excretion loading	0.48	0.48	-0.68

FIGURES

Fig. 1: Percent of dry weight in phosphorus (A) and log-transformed P excretion (B) of guppies from high predation (HPred, dark symbols) and low predation (LPred, light symbols) environments and each of two rivers (Aripo = blue shades; Guanapo = orange and yellow) fed diets of high and low phosphorus content (x-axis). Both tissue nutrient and nutrient excretion declined for guppies fed a low P diet. Large symbols are treatment means with standard error bars. Small symbols are individual fish in each treatment group. Some variation in the location of points on the x-axis has been added to facilitate comparison of values.

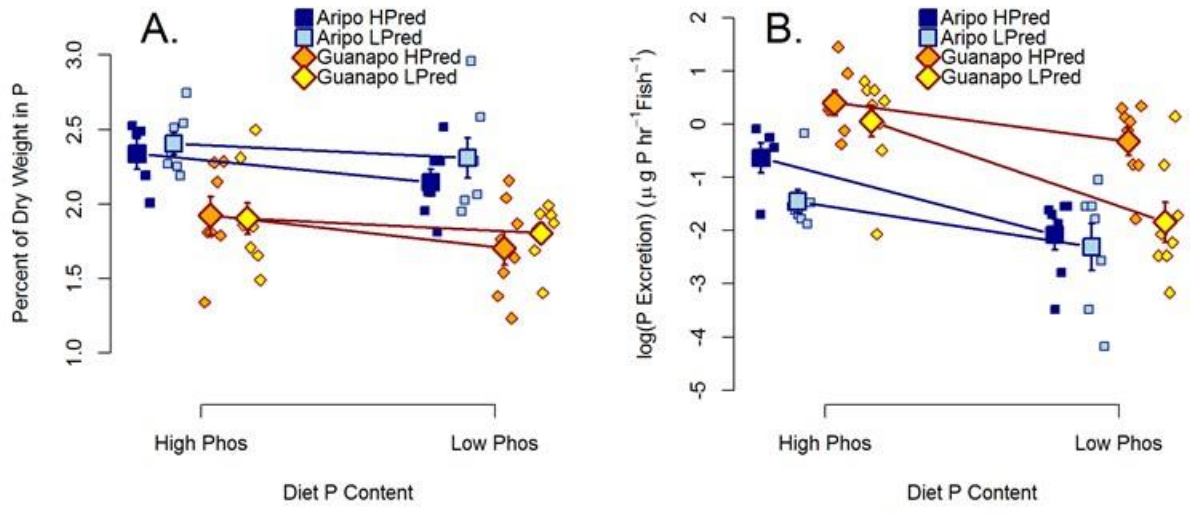


Fig.2: Treatment and population mean P excretion as a function of treatment and population mean tissue %P. Shown here are high predation ("HPred") and low predation ("LPred") guppies from the Guanapo ("Gu.") and Aripo ("Ar.") Rivers on the high P diet (black symbols and lines) and low P diet (gray symbols and lines). Lines represent best fit linear models for population means on the high P diet (black solid line) and the low P diet (dashed gray line). Population mean excretion declines with population mean tissue P content, but this relationship is confounded by the positive effect of diet on both tissue and excretion nutrients for any given population (*i.e.*, compare light and dark shades for any given color-shape combination).

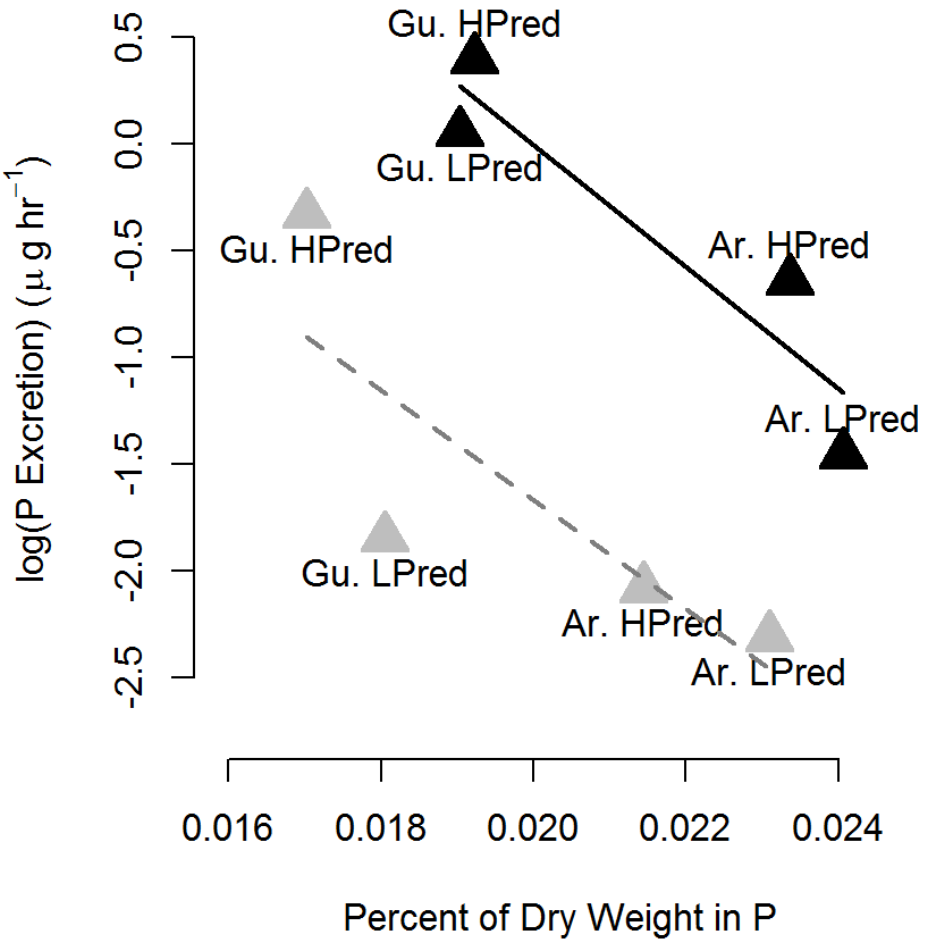


Fig. 3: Principal components analysis of guppy stoichiometric traits in this experiment. (A) All 58 guppies in the experiment. The first axis of variation (PC1), generated almost entirely by tissue nutrient concentrations, is almost entirely unrelated to ancestral predation environment (HPred = Blue circle or square; LPred=Red diamond or triangle) or diet phosphorus content (Dark = HiPhos; Light = LoPhos). The second axis of variation, generated primarily by nutrient excretion, is higher for HPred than LPred and higher for HiPhos than LoPhos. HPred LoPhos and LPredHiPhos arrows are difficult to detect, as the magnitude of their loading is very small. Similar patterns are present for (B) Aripo guppies and (C) Guanapo guppies, which are distributed along the left (Aripo) and right (Guanapo) regions of the x-axis. Note that in (B) the HPred LoPhos guppies have comparable loadings as the two LPred treatments, making the loading of this arrow difficult to detect. Excretion nutrients are largely decoupled from tissue nutrients, as evidenced by their largely orthogonal loadings. Light gray arrows represent the loadings of each stoichiometric variable onto the analysis. Thick colored arrows represent the mean PC scores of each treatment group.

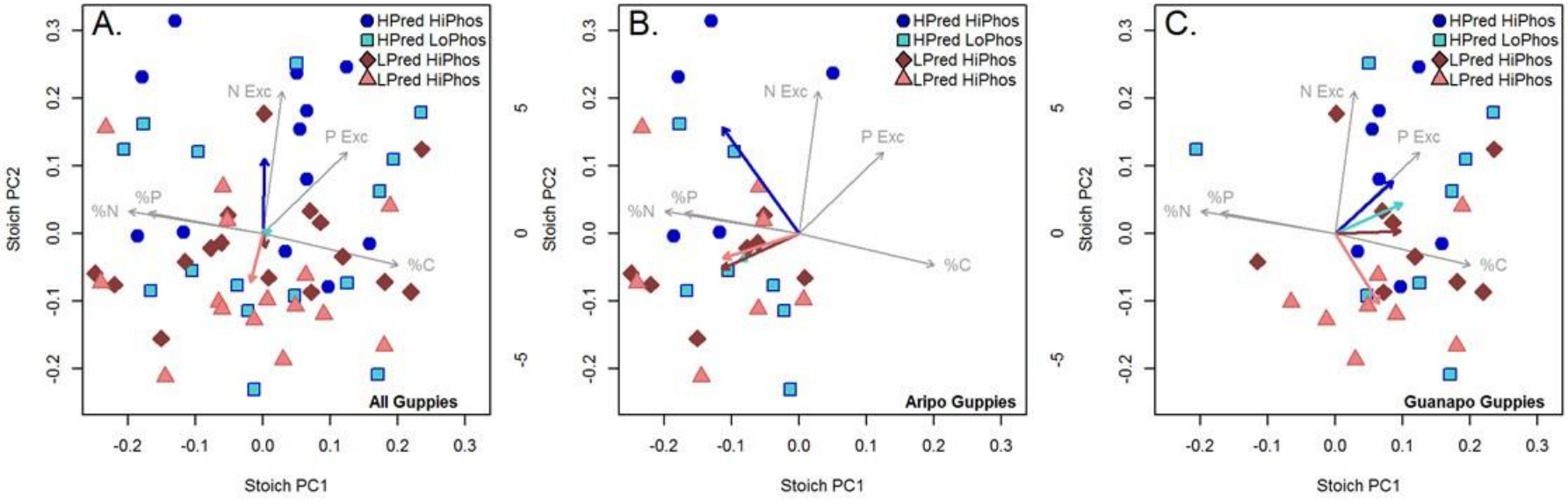
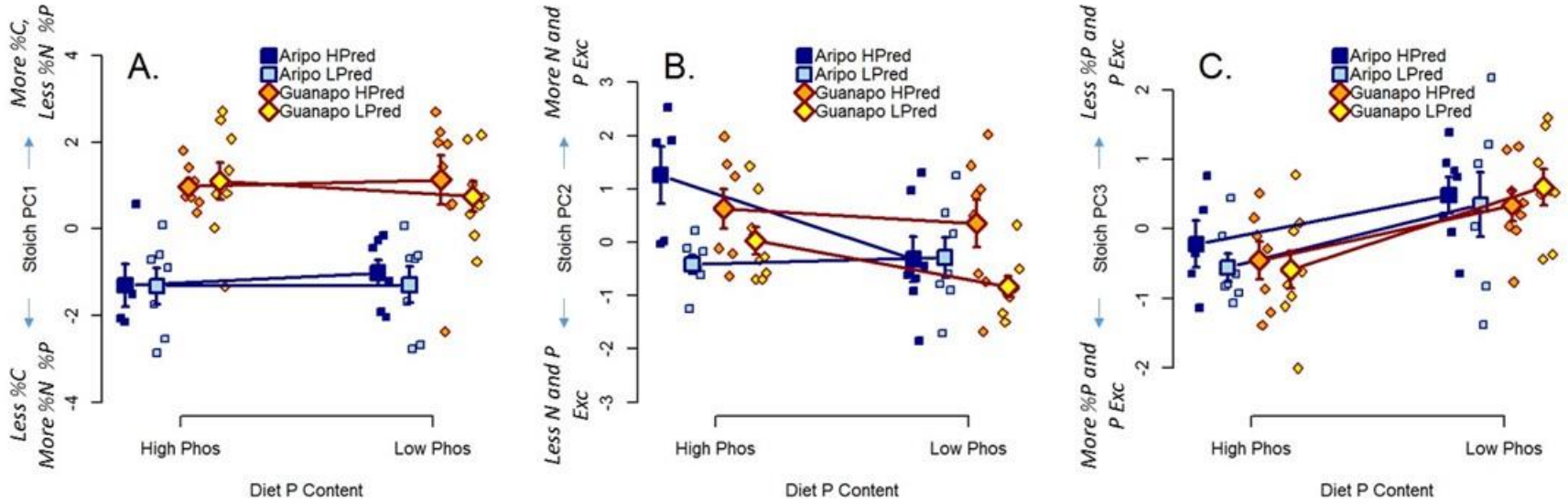


Fig. 4: Principal Component Analysis scores results for PC1 (A), PC2 (B), and PC3 (C) for HPred (dark symbols) and LPred (light symbols) from the Aripo (blue squares) and Guanapo (orange -yellow diamonds) on a high and low P diet. (A) Diet treatment and ancestral predation environment did not affect PC1, which reflected mostly tissue composition. Guanapo guppies had higher PC1 scores, reflecting their higher tissue C and lower tissue N and P. This effect may be a result of the difference in size during the experiment (Guanapo guppies were larger). (B) Both diet and ancestral predation environment affected PC2, which reflects N and P excretion. HPred guppies and guppies on the high P diet had higher PC2 scores than LPred and low P diet guppies. (C) PC3, which inversely reflects tissue and excretion P content, was increased in all populations by the diet treatment, reflecting the parallel influence of diet on tissue and excretion P. Large symbols are treatment means with standard error bars. Small symbols are individual fish in each treatment group. Some variation in the location of points on the x-axis has been added to facilitate comparison of values.



CHAPTER TWO: TOP-DOWN AND BOTTOM-UP INFLUENCES ON THE STOICHIOMETRIC TRAITS OF A GENERALIST CONSUMER

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ABSTRACT

Consumers can change ecosystems by storing limiting nutrients in tissues, by releasing limiting nutrients as byproducts of metabolism, or both. Describing causes of variation in consumer nutrient budgets is thus central to understanding ecological dynamics. Fish can be important nutrient recyclers in many aquatic ecosystems, and, though researchers have developed effective frameworks to describe interspecific variation in fish nutrient budgets, these frameworks fail to describe most intraspecific variation in fish-mediated nutrient cycling. Here, we use a field survey to contrast traditionally-assessed relationships between excretion rate and tissue or dietary nutrients with a potentially important but little studied source of variation: predation risk. We surveyed tissue and excretion nutrients of Trinidadian guppies (*Poecilia reticulata*), as well as guppy diets and resource abundance, across independent gradients of light and predation risk gradients in two Trinidadian streams. In line with previous studies, we found that guppy tissue nutrients were well-predicted by bottom-up influences. Guppies from high resource environments were in better physiological condition, as evidenced by greater tissue C and N content. Like previous studies, we found only weak evidence for bottom-up influences on guppy excretion and no evidence for a relationship between tissue and excretion nutrients. Our analysis, however, indicated that guppy excretion was primarily affected by the strength of predation risk. Guppies living under predation risk had emptier guts and lower excretion rates than guppies from predator-free environments. The independent influences of resources on tissue nutrients and predation risk on excretion nutrients decoupled excretion from body tissue composition. We suggest predation risk may be a central cause of variation in consumer-mediated nutrient cycling.

INTRODUCTION

Consumer-mediated nutrient cycling is central to understanding and predicting ecological dynamics (Capps et al. 2015). Consumers can serve as sources or sinks for limiting nutrients (Vanni et al. 2013), thus exacerbating or alleviating nutrient limitation in ecosystems from coral reefs to old fields (Hawlena and Schmitz 2010a; Layman et al. 2013). These consumer-driven nutrient cycling effects are especially pronounced in aquatic ecosystems, where inverted trophic pyramids can put the largest pools of organic nutrients at high trophic levels (*e.g.*, (Sandin et al. 2008; Wang et al. 2009)). Here, fishes are often the dominant taxa, and variation in the rates and elemental ratios of fish excretion can transform nutrient dynamics in many ecosystems (Vanni 2002; Capps and Flecker 2013).

Early studies on nutrient recycling by fish emphasized differences between species (Vanni et al. 2002; McIntyre et al. 2007; McIntyre and Flecker 2010). These studies found patterns that supported existing paradigms for variation in consumer-mediated nutrient recycling: interspecific variation in nutrient excretion was directly proportional to dietary nutrients and inversely proportional to tissue nutrients (Vanni et al. 2002; McIntyre and Flecker 2010). These results suggest that variation in fish excretion could be predicted simply by measuring the diet and tissue nutrients of fish (*i.e.*, excretion nutrients = diet nutrient content – tissue nutrient content), yet studies on intraspecific variation in fish nutrient budgets suggest otherwise.

Field surveys of variation within a single species have often failed to detect positive associations between fish excretion and dietary nutrients ((McManamay et al. 2011; Villéger et al. 2012); but see (Higgins et al. 2006; Torres and Vanni 2007)), and no published study has found correlations between excretion and tissue nutrients in any single fish species ((Higgins et al. 2006; Torres and Vanni 2007)). Because intraspecific variation in nutrient budgets can be both substantial (Jeyasingh et al. 2014) and important for ecosystem function (Bassar et al. 2010;

Bassar et al. 2012; Taylor et al. 2012b), researchers must ponder whether intraspecific variation in fish excretion is simply intractable or related to a previously neglected cause of variation.

Recent empirical evidence suggests predation risk is a rarely-considered but highly influential cause of intraspecific variation in excretion. Studies in terrestrial food webs indicate predation risk induces change in the metabolic stoichiometry of prey by triggering a general stress response that may be general among animals (Hawlena and Schmitz 2010a). This altered stoichiometry can drive predictable and dramatic change in the nutrients that are recycled in consumer wastes and stored in consumer tissues (Hawlena and Schmitz 2010a), leading to change in ecosystem function (Hawlena et al. 2012; Leroux et al. 2012). Studies in aquatic systems have also linked predators to variation in the nutrient budgets of consumers through changes in their functional morphology (Costello and Michel 2013), feeding behavior, and metabolic stoichiometry (Dalton and Flecker 2014). Here, we survey consumer nutrient cycling along a gradient in predation risk, using measures of diet and resource availability to contrast top-down and bottom-up influences on tissue and excretion nutrients.

Our study species is the Trinidadian guppy (*Poecilia reticulata*), which inhabit streams with variable predator assemblages. Some Trinidadian stream reaches have abundant predators that impose high extrinsic mortality on guppy populations, while other stream reaches entirely lack effective piscivores (Reznick et al. 1996a). Guppy life histories have repeatedly evolved under this variation in predation mortality, with guppies in high predation risk (HP) sites showing higher reproductive output of more, smaller offspring, earlier in life than do guppies from low predation risk (LP) sites (Reznick et al. 1996b).

Variation in predation risk occurs in concert with gradients in light availability that alter the availability of resources and influence guppy growth rates (Grether et al. 2001; El-Sabaawi et

al. 2015a). Manipulative experiments indicate that predation and resource availability affect guppy tissue and excretion nutrients. HP guppies reared with chemical cues from predators have lower rates of N excretion than guppies reared without predator cues (Dalton and Flecker 2014). In the absence of predation risk, however, guppies from populations co-evolved with predators have higher excretion rates than do guppies locally adapted to predator-free environments (Palkovacs et al. 2009; Bassar et al. 2010). Resources can also influence guppy excretion rates, as HP and LP guppies experimentally introduced to higher resource environments have higher N and P excretion than those introduced to low resource environments (El-Sabaawi et al. 2015a).

Guppies are thus a compelling model for assessing top-down and bottom-up drivers of variation in nutrient recycling. Here, we present the results of a field survey that assessed guppy tissue and excretion nutrients along gradients in resource environment and the presence of a dominant diurnal predator, the pike cichlid, *Crenicichla frenata* (hereafter, *Crenicichla*). The goal of this study is to assess the factors dictating guppy stoichiometric traits as a model for understanding intraspecific variation in fish stoichiometry.

METHODS

Study Sites

We surveyed guppy resources, diet, and stoichiometric traits in two rivers in the Northern Range Mountains of Trinidad and related these variables to two independent gradients: (1) a top-down ecological gradient in predation environment (Gilliam et al. 1993; Bassar et al. 2010) and (2) a bottom-up gradient in light availability. Six sites in each of the two rivers were selected to span from predator-free headwater streams to main-stem localities with diverse predator assemblages.

Sites were arrayed along two different rivers in the Caroni River drainage (Aripo and Guanapo Rivers), that vary in their barriers to upstream fish dispersal. The Aripo River (and predator-free Naranjo tributary) is marked by a series of karst waterfalls that entirely restrict predator access to upstream reaches of even the mainstream river. This structure creates marked differences in fish assemblages above and below these barrier waterfalls. The Guanapo River is marked by less restrictive gorges and small waterfall plunges that cause predator species to slowly drop out of the community with distance upstream, with only headwater streams containing piscivore-free conditions (Gilliam et al. 1993).

Within each river drainage, six widely separated sampling sites were selected along the gradient from highest to lowest predation environment. At each sampling site, three main-stem pools were selected for this study. Pools varied in their physical, chemical, and biological composition (Table 1), with the methods used to capture this variation described below. Using "pool" as the main level of analysis reflects the population biology of guppies, as very few guppies migrate among pools within a generation (Reznick et al. 1996a). Each pool was assessed for its canopy cover using methods described by Zandona et al. (Zandonà et al. 2011), and predation environment was determined based on direct observation of the fish community as well as reference to previous surveys of fish assemblages along these stream reaches (Gilliam et al. 1993; Bassar et al. 2010; Zandonà et al. 2011). All sampling was conducted from 27 March to 9 April in 2011, between 09:00 and 16:00.

Resource environment

Epilithon composition was sampled following the methods in Kohler et al (2012). In brief, at each pool, three representative rocks from habitats in which guppies were observed were collected and scrubbed with a toothbrush to remove epilithon. This slurry was stored in an Whirl-

Pak ®, transported on ice, then homogenized and filtered within several hours onto 25 mm and 37 mm GF/F for analysis of chlorophyll *a* and ash-free dry mass (AFDM). The surface of each rock was traced in the field, and tracings were photographed in the laboratory and analyzed with ImageJ software (Abramoff et al. 2004) to measure total rock area. AFDM was quantified as the difference in the dry weight of filters before and after ashing at 500 °C for 4 h. Chlorophyll *a* was assessed by fluorometric analysis of overnight extractions of filters in buffered 90% ethanol as in Dalton et al. (2012). Benthic organic matter (BOM) and invertebrate densities were estimated by isolating a known area of benthic area using a PVC sleeve, collecting all organic matter in this sleeve (or 1 L of organic matter for BOM), and filtering the organic matter-following the methods of Bassar et al . (2012) and Zandonà et al. (2011), respectively.

Guppy diet

In each pool, five adult female guppies were selected haphazardly from amongst more than 20 guppies captured from all representative habitats in the pool. Guppies were euthanized using ice water, immediately placed on ice and transported to the lab for storage at -10 °C until analysis. All guppies were measured for length and weight immediately prior to gut dissection. Complete guppy gastrointestinal tracts were then dissected and analyzed for length and content following the methods of Zandonà et al (Zandonà et al. 2011). Dissected guts were preserved in 5% formalin solution until analysis. For diet analysis, the contents of individual guppy foreguts were placed on 64 cell gridded slides, and the area of the slide covered by each of five diet categories (detritus, filamentous algae, diatoms, invertebrates) was quantified using a random sampling procedure. The number of grid cells where no diet items were present was also counted as a measure of gut emptiness.

Guppy stoichiometry

In one pool at each sampling access, ten intermediate-sized adult female guppies (the largest and smallest females at a given site were intentionally avoided) were selected haphazardly from a set of guppies collected from all representative habitats within the pool. These guppies were used for estimation of excretion and tissue nutrients. Excretion methods were as in (El-Sabaawi et al. 2015b), except that (1) nutrient measurements were only taken after a 20-minute acclimation period to minimize effects of handling stress while avoiding fasting-based inhibition of excretion (Whiles et al. 2009), (2) incubations were set at 60 minutes to capture longer-term averages in excretion rates, and (3) guppies were incubated in containers situated in the stream water itself, both reducing unnatural stressors and avoiding temperature fluctuations. Samples for nutrient analysis were immediately filtered with 25 mm GF/F Whatman filters (pore size = 0.7 µm), placed on ice, and analyzed for ammonium (NH_4^+) and soluble reactive phosphorus (SRP) concentration using the methods of Taylor et al. (2007) for NH_4^+ and the molybdate-antimony method for SRP (Parsons et al. 1984). Hourly excretion was estimated as the difference in nutrient concentration between the two samples divided by the length of the incubation, in hours, after correcting for the volume of water in the container during the incubation.

At the conclusion of an excretion measurement, guppies were sacrificed in ice water, placed on ice, and frozen to -10°C within 5 h. For body nutrient analysis, guppy digestive tracts were dissected and discarded, and the combined somatic and reproductive tissues of each guppy were dried to constant mass at 55°C. Dried guppies were then weighed and ground into a homogeneous powder using a Wig-L Bug® sample grinder. Subsamples (2 mg for C and N, 20-40 mg for P) of guppy tissue were then assayed for C, N and P content following methods described in El-Sabaawi et al (2012b). Briefly, 2 mg subsamples of homogenized tissue were

weighed to the nearest 0.001 mg, and the percent carbon (C) and nitrogen (N) of each sample was assayed (Vario EL III elemental analyzer, Elementar, Hanau Germany). The remainder of the guppy tissue was weighed, ashed in Pyrex vials at 500°C for 2 h and digested in 1N HCl at 105°C to facilitate dissolution of P. SRP of the resulting solution was then quantified using serial dilutions and the molybdate-antimony method, with spinach and bone meal used as quality control standards.

Statistical analyses

Data on resources and guppy diets were analyzed for each of three pools nested within each of six access points within each of two rivers. These data were analyzed using pool-level averages, and the non-independence of pools within each sites was modeled by including the site ID as a random effect in every model (river was included as a fixed effect, with interactions with all main effects). Guppy diets were analyzed for individual guppies, with both pool ID and site ID used as random effects to account for the non-independence of guppies from the same pool and pools from the same access.

Data for guppy stoichiometric traits were only collected in one pool per access, and individual guppy data were analyzed with access ID as a random effect to account for non-independence of fish from the same access. Size was a significant predictor of both N and P excretion, but no size × resource or size × predation environment interactions were significant. As a result, we analyzed variation in size corrected excretion rates, which were calculated by accounting for allometry as in Torres and Vanni (2007).

Principle components analysis was conducted on each category of variables (*e.g.*, resource, diet, stoichiometry) after log-transforming (where necessary), centering, and scaling

each variable. All analyses were conducted in R (R-Team 2010), using the packages lme4 and prcomp to run linear mixed models and principle component analysis, respectively.

RESULTS

Bottom-up and Top-down Influences on Guppy Resources

Our measures of top-down and bottom-up control, predator presence and canopy cover (proxy for light availability) (respectively), were not confounded (Supplemental Table 1), enabling assessment of the independent influence of predators and light availability on stream resources. However, different measures of guppy resources were confounded, as pool-average chlorophyll *a* and epilithon biomass were correlated ($t = 2.153$, $p = 0.0389$) and pool-average invertebrate density and detrital biomass were correlated ($t = 2.794$, $p = 0.009$). Analysis of predators and light effects on each individual resource stock can be found in the supplemental materials. (Supplemental Materials, Tables 2-5), but are not discussed in detail here.

We used a PCA to partition 72% of the variation in four measures of guppy resources onto two component axes (Figure 1A, Figure 1B; Table 2). PC1 explained 39% of the variation in resources and was generated by positive loadings from all resource measures. PC2 explained 33% of the variation in resources and was generated by positive loadings from invertebrates and detritus and negative loadings from epilithon AFDM and chlorophyll *a*.

Sites with predators had more of all resources (*i.e.*, higher PC1 scores), and canopy cover increased resource abundance in the absence of predatory fish (Figure 1C, Table 3A). Resource PC1 was positively related to canopy cover in sites without predators (slope estimate = 0.046 ± S.E. = 0.023), and sites with predators had higher PC1 scores (estimate = 2.373 ± S.E. = 0.685) that were not affected to canopy cover (estimate = -0.007 ± S.E. = 0.018) (Figure 1C). Removing the canopy × predation interaction significantly reduced model explanatory power (likelihood

ratio test (LRT): df = 1, χ^2 = 3.851, p = 0.0497). These fixed effects explained a substantial portion of the variation in Resource PC1, as this best model had a marginal R^2 of 0.39.

The relative abundance of invertebrates and detritus compared to epilithon (PC2) was not influenced by river or predation risk, but Aripo sites without *Crenicichla* had significantly lower PC2 scores (estimate = -1.652 ± S.E. = 0.676; Figure 1D, Table 3B). Removing the predation × river interaction effect significantly reduced model explanatory power (df = 1, χ^2 = 5.450, p = 0.020). No model with canopy cover received substantial support for explaining variation in Resource PC2. The best model for Resource PC2 (with a river × predation interaction) had a marginal R^2 of 0.29.

Resource availability and Predation environment Influences on Guppy Diet

Diatoms and other algae never made up more than 5% of guppy diets and were excluded from the analysis. As expected, dietary components were confounded (Table 4). Dietary invertebrate content and gut empty space were positively correlated (t = 2.22, p = 0.028), and dietary detritus content was negatively correlated with both dietary invertebrates and gut empty space (diet invertebrates: t = -4.86, p <0.001; diet empty space: t = -9.803, p < 0.001). Analysis of individual diet items can be found in the supplemental materials (Supplemental Materials, Tables 6-8), but are not discussed here further.

Instead, we used a principle components analysis of diet data to partition 88% of the variation in guppy diets onto two axes (Table 5; Figure 2A and Figure 2B). PC1, which explained 60% of the variation in diet, was generated by positive loading from both diet invertebrate area and empty space and by negative loading from the amount of detritus in the gut. This axis thus differentiates guts that were filled with mostly detritus (low quality food) from all other guts. PC2, which explained 28% of the variation in diet, was generated by negative loading

by the emptiness of the gut and positive loading of the invertebrate area, with slight positive loading from the gut detritus area. Of guppies with positive PC1 scores (*i.e.*, low detritus consumption), this axis differentiates guts that had large quantities of invertebrates from guts that contained fewer invertebrates and thus were emptier. We assessed the influence of resource availability and predator preence on these two axes of variation in the diet by comparing models for dietary PC1 and PC2 that had main effects of predation (presence of *Crenicichla*) and resource availability (Resource PC1 and PC2) and all two way interactions.

Consumption of invertebrates by guppies increased with invertebrate density. Diet PC1, which differentiated invertebrate-filled or empty guts from detritus-filled guts, increased with Resource PC2, which differentiated invertebrate-dominated sites from algal dominated sites (slope estimate = 0.184 ± S.E. = 0.101; Figure 2C, Table 6A). Diet PC1 was also significantly higher in Aripo sites with predators (estimate = 1.303 ± S.E. = 0.535). Removing the resource PC2 or river × predation terms significantly reduced model explanatory power (LRT: Resource PC2: df = 1, χ^2 = 3.993, p = 0.0457; river × predation: df = 1, χ^2 = 6.937, p = 0.008). These terms explained 25% of the variance in Diet PC1.

Diet PC2, which differentiated guppies with invertebrate filled guts from guppies with empty guts, was best explained by Resource PC2 (Figure 2D, Table 6B), but this model had a marginal R^2 of only 0.02. Guppies at sites with higher Resource PC2 scores had higher Diet PC2 scores (slope estimate = 0.125 ± S.E = 0.062). Guppies in the Guanapo had higher Diet PC2 scores (estimate = 0.230 ± S.E. = 0.145), but a model with only the effect of Resource PC2 received comparable support, and removing the River term did not significantly reduce model explanatory power (LRT: df = 1, χ^2 = 2.382, p = 0.122). A model with river, predator presence, and a Resource PC2 × predator presence interaction also received substantial support, but

removing the two terms containing predator presence also did not significantly reduce model explanatory power (LRT: df = 2, χ^2 = 4.154, p = 0.125).

Because higher Resource PC2 scores are associated with high invertebrate density and low epilithon stocks, the positive correlation between Diet PC2 and Resource PC2 indicates that guppies at sites with more abundant invertebrates have fewer empty, and more invertebrate-filled guts, though nearly all of the variation in Diet PC2 remained unexplained by resources, river, or predation environment.

Diet, River, and Predation Effects on Tissue and Excretion Nutrients

Guppy tissue nutrients and excretion nutrients were highly confounded (Table 7). The percent carbon (C), nitrogen (N), and phosphorus (P) of guppy tissues was tightly correlated, positively for C and N (t = 6.59, p < 0.001) and negatively for C and P (t = -12.51, p < 0.001) and for N and P (t = -5.94, p < 0.001). N and P excretion were also tightly correlated (t = 5.40, p < 0.001). To assess variation in tissue and excretion stoichiometry, we thus used principle components analysis of the three measures of tissue nutrient composition (percent of dry weight composed of C, N, and P) and two measures of excretion (size-independent N and P excretion). Analysis of individual nutrient measures can be found in the supplemental materials (Supplemental Materials, Tables 9-13), but are not discussed at more length here.

We used a principle components analysis to decompose 76% of the variation in tissue and excretion nutrients onto two axes (Table 8). PC1 comprised 46% of the variance in tissue and excretion nutrients and was generated by positive loading from tissue %C and %N and negative loading from tissue %P and slightly from P excretion (Table 8, Figure 3A and 3B). Nutrient PC2 comprised 30% of the variance in tissue and excretion nutrients and was generated primarily

from negative loading by N and P excretion, with minor positive loading from tissue %P and minor negative loading from %C.

These results highlight that tissue and excretion nutrients are largely independent of each other: PC1 was generated primarily by loading from tissue nutrients, and PC2 was generated primarily by loading from excretion nutrients. Loading on PC1 also highlights the inverse relation between tissue C or N and P, which may reflect dilution of relatively inflexible skeletal P by guppies rich in C-intensive lipids and N-intensive proteins. We assessed top-down and bottom-up influences on tissue nutrients by comparing models for Nutrient PC1 and PC2 that had main effects of predation (presence of *Crenicichla*) and diet (Diet PC1 and PC2) and all two way interactions.

Nutrient PC1 was best explained by Diet PC1, river, and a River × Diet PC1 interaction (Table 9A, Figure 3C). This model had a marginal R^2 of 0.48. Nutrient PC1 scaled positively with Diet PC1 (invertebrates vs. detritus) in the Aripo River (slope estimate = 0.879 ± S.E = 0.318), was higher in the Guanapo (intercept difference estimate = 2.322 ± S.E. = 0.432) and scaled more steeply with Diet PC1 in the Guanapo (slope estimate = 1.873 ± S.E. = 0.620). Other models for Nutrient PC1 received substantial support, though all included an effect of Diet PC1, and adding additional terms did not significantly improve model explanatory power, while removing the river × Diet PC1 interaction marginally reduced model explanatory power (LRT: df = 1, χ^2 = 2.88, p = 0.0895). Higher values for Nutrient PC1, which indicate higher tissue C and N and lower tissue P, are thus associated with higher quality invertebrate-filled (and less detritus-filled) diets that are reflected by Diet PC1.

In contrast, Nutrient PC2, which mostly reflects excretion, was unrelated to diet quality. The best model for Nutrient PC2 included only an effect of predation environment (Table 9B,

Figure 3D), with Nutrient PC2 being higher (reflecting lower excretion) in sites with predators. This model had a marginal R^2 of 0.34. A model with an additional Diet PC2 term also received substantial support (Table 6B), but removing this effect did not significantly reduce model explanatory power (LRT: df = 1, χ^2 = 1.190, p = 0.275). Guppy excretion nutrients, then, are best explained by the presence of predatory fish and are independent of diet quality.

DISCUSSION

Our results indicate top-down and bottom-up forces affected the tissue and excretion nutrients of a generalist consumer. Guppy tissue nutrients reflected resource-driven dietary patterns, while guppy excretion nutrients were best explained by the presence of predatory fish. Bottom-up and top-down influences acted independently, decoupling patterns of tissue nutrients from patterns of excretion nutrients. The goal of this study was to describe the environmental controls on variation in the tissue and excretion nutrients of guppies, using guppies as a model for intraspecific variation in general. Here we focus on guppies as the object of environmental variation, and further study in the field would be necessary to assess the importance of these variables for ecosystem processes in this system.

Organismal and Ecological Influences on Guppy Tissue Stoichiometry

Carbon, nitrogen and phosphorus concentrations in guppy tissues were highly confounded (Figure 3A), at least in part due to "percent of dry mass" metrics. Such metrics, while widespread in stoichiometric literature, are often confounded because the denominator of this metric includes the mass of all other elements (*e.g.*, %P = (mass of P) × (mass of C + mass of N + mass of P + mass of all other elements)$^{-1}$). Thus, an increase in the mass of one element in an organism (for example, tissue %C or %N) will necessarily decrease the concentration of all other elements, even if the masses of those elements remain unchanged. We have addressed this

issue by using a principle components analysis of all measured tissue and excretion nutrients to collapse covariance in tissue and excretion nutrients onto the most influential axes of variation.

The first axis in this principle components analysis reflects variation in tissue nutrients, as stoichiometric PC1 was generated by positive loadings from tissue %C and %N and negative loadings from tissue %P (Figure 3A and 3B) with little influence of guppy excretion nutrients (Table 5). The correlation between Nutrient PC1 and Diet PC1 (Figure 3A) indicates guppies with high tissue %C and %N (and low tissue %P) had more invertebrate rich (and less detrital-based) diets (Figure 3C). Diet PC1, moreover, was positively related with the abundance of invertebrates in the environment (Resource PC2; Figure 2C and Table 6A). Thus, guppies from sites with high invertebrate abundance had a high invertebrate content diet and high tissue %C and %N and low tissue %P.

Because invertebrates are a high nutrient food (El-Sabaawi et al. 2012b), and because high tissue %C and %N generally reflect fish in good physiological condition (McIntyre and Flecker 2010), we see this result as evidence of bottom-up forcing on guppy tissue stoichiometry. Better resource environments led to better guppy diets, which induced higher tissue protein and lipid content, as reflected in tissue %C and %N. This bottom-up forcing on tissue nutrients is comparable to a pattern that has been detected in the guppy competitor, *Rivulus hartii*, in some of these same streams (El-Sabaawi et al. 2012a).

The correlation between Nutrient PC1 and Diet PC1, however, also links detritus consumption (low Diet PC1) and tissue %P (low Nutrient PC1). This elevated tissue %P in more detritivorous fish may occur as a result of selection imposed by a detritivorous diet. Indeed, some of the world's highest P content fishes consume detrital foods that nearly completely lack available P (Hood et al. 2005; McIntyre and Flecker 2010); moreover, our lab studies have

demonstrated that guppies from detritus-dependent populations have higher tissue %P, even when reared on standardized diets for one or more generations (Sullam et al., 2014). The correlation between high dietary detritus content (low Diet PC1 scores) and high tissue %P (low Nutrient PC1 scores) may thus be related to some form of local stoichiometric adaptation driven by a low-P, high detritus diet, but the adaptive significance of this pattern remains untested.

The association between detrital diets and high tissue P, however, may more parsimoniously be attributed to the relationship between food quality and tissue C and N. If higher quality foods increase the mass of C and N in guppy tissues without affecting the largely skeleton-derived mass of P, then the concentration of P would necessarily decline. Indeed, we achieved this effect by feeding lab-reared guppies from the Aripo River a low-quality diet that mimicked the quality of detritus. This low-quality diet lowered guppy physiological condition and tissue C, increasing the *concentration* of guppy tissue P without actually increasing the *amount* of P in guppy tissues (Sullam et al. 2014). Similarly, a field and lab-based study accomplished similar results by increasing bluegill tissue N content with supplemental feeding, reducing the concentration of P in tissues as a result (Glaholt and Vanni 2005). Variation in the tissue P content of adult fish is explained almost entirely by variation in tissue skeletal mass (Hendrixson et al. 2007). Dilution of this relatively inert skeletal-P by variable stocks of lipid-C and protein-N may be the primary cause of variation in tissue P in our field dataset and is likely a general cause of intraspecific variation in tissue P content.

This flexibility in tissue nutrients with diet quality runs counter to the notion of strict tissue homeostasis (Sterner and George 2000; McManamay et al. 2011), and, in fact, we doubt that strict homeostasis occurs very often in fish. For example, fish tissue %C varies substantially with the size of lipid stores that vary with the energy content of fish diets (McConnaughey and

McRoy 1979; Post et al. 2007), and changes in fish tissue %P can be induced by manipulating diet nutrient content in the lab (Benstead et al. 2014). Despite the near certainty of diet-induced variation in fish tissue nutrients, few field surveys assess how diet quality affects tissue stoichiometry (Tanner et al. 2000; Higgins et al. 2006; Dantas and Attayde 2007; Hendrixson et al. 2007). Studies that do explicitly test for dietary effects on tissue nutrients routinely find positive relationships between diet quality and tissue nutrients (Vrede et al. 2011; El-Sabaawi et al. 2012a). Our result is concordant with this finding. The tissue nutrients of guppies in Trinidad were best explained by the quality of guppy diets. Whether this difference relates to dietary effects on morphology (*i.e.*, altered skeletal structure (Hendrixson et al. 2007)) or physiological condition (*i.e.*, altered lipid and protein stores (McIntyre and Flecker 2010; Dalton and Flecker 2014)) is a question deserving more empirical attention.

Drivers of variation in guppy nutrient excretion

Size-independent measures of N and P excretion varied among our sampling sites by factors of 2.8 and 14.7, respectively. The magnitude of this variation highlights the importance of understanding causes of intraspecific variation in excretion rates, but this degree of variation was not unexpected. Previous studies have shown dramatic variation in nutrient excretion by different populations of fish, such as a 59% change in community-wide nutrient recycling resulting from change in excretion rate by a single species of fish (Sereda et al. 2008).

Most often, earlier studies have emphasized the role of bottom-up influences on variation in fish excretion rates. Field studies have correlated excretion rates with diet nutrient composition or gut fullness (Higgins et al. 2006; Small et al. 2011), and mesocosm studies consistently show bottom-up influences of food quantity or quality on fish excretion (Glaholt and Vanni 2005; Palkovacs et al. 2009; Bassar et al. 2010; Taylor et al. 2012a; Taylor et al. 2012b;

El-Sabaawi et al. 2015a). Field studies, nonetheless, leave substantial portions of this important variation in nutrient excretion unexplained (Villéger et al. 2012).

Our results link variation in guppy excretion with the predation environment of a given site, independent of the resources available at that site. We are unaware of any other system in which predation risk has been tested as a driver of variation in excretion by fish, yet our results point to predation risk as the factor that most strongly drives variation in guppy nutrient excretion. Guppies in sites with predators had higher Nutrient PC2 scores (Figure 3D), indicating *lower* excretion rates of both N and P by individual guppies in pools where predators were present.

This pattern runs counter to a previously-published estimate of differences in excretion rates among guppies from sites with and without predators (El-Sabaawi et al. 2015b). This difference may arise from the difference in our sampling design, which emphasizes replication within, instead of across, rivers. Notably, as well, our excretion rates were collected with considerable effort to minimize stress during the incubation, longer incubation times, use of an acclimation period, naturalistic incubation conditions, and the lack of any disturbance during the course of the incubation. Direct comparison of these methods in the field, however, would be necessary to assess their influence. Interestingly, our pilot lab studies suggested that HP guppies may respond more strongly to incubation stress than LP, potentially explaining the reversal in results between the two studies.

This reduction in excretion in sites where predators occur may be underlain by reduced feeding frequency by guppies under predation risk. Such reduced feeding by guppies under predation risk has previously been demonstrated in both field and lab studies (Fraser and Gilliam 1987; Fraser et al. 2004; Torres-Dowdall et al. 2012) and has been correlated with reduced

excretion rates in lab-reared guppies (Dalton and Flecker 2014). Indeed, feeding spurs digestive metabolism and creates surges of assimilated nutrients in the bloodstream that increase fish N and P excretion rates following a meal (Lyndon et al. 1992; DeSilva and Anderson 1995; Luo and Xie 2009). For example, our lab studies indicate that guppies excrete 3.7 and 4.1 times more N and P (respectively) immediately after feeding than after several hours of fasting (Dalton & Flecker, 2014; C. Dalton unpub. data).

Because diurnally active predators, like *Crenicichla*, reduce feeding by guppies during the day, lower rates of excretion in sites with predators may reflect the longer duration of time since that guppy's previous meal. Indeed, guppies in sites with predators had emptier guts than those in predator-free sites (lower Diet PC2 scores). The emptiness of guppy guts, moreover, was a good predictor of guppy excretion rate, as guppies with emptier guts (lower Diet PC2 scores) had marginally lower nutrient excretion rates (higher Nutrient PC2). This result of lower N intake (consumption) and lower excretion is concordant with our lab research (Dalton and Flecker 2014), which has shown that guppies in water with *Crenicichla* cues consume less food and excrete proportionately less N than guppies in water without these cues, causing predation risk to substantially reduce guppy-driven nutrient recycling.

Predation risk and feeding behavior are rarely considered in field studies of variation in fish excretion, but may be widespread drivers of variation in this important component of freshwater nutrient cycles. Indeed, researchers in terrestrial ecosystems have demonstrated dramatic effects of predators on the metabolic and tissue stoichiometry of their prey that may be general across many species of consumer (Hawlena and Schmitz 2010b; Hawlena and Schmitz 2010a), with changes in prey stoichiometry translating into changes in ecosystem function (Hawlena et al. 2012; Leroux et al. 2012). We suggest that these factors may contribute to the

often substantial among-population variation in excretion that cannot be explained by more traditionally-considered influences on fish excretion (*e.g.*, (McManamay et al. 2011; Villéger et al. 2012)).

Comparing tissue and excretion nutrients

Our results indicate guppy nutrient excretion is decoupled from tissue nutrient content, as tissue N and P concentrations explained just 0.3% and 2.4% of variation in guppy N and P excretion (respectively). Nutrient PC1 and PC2 scores also suggest this decoupling. Loading of tissue nutrients onto Nutrient PC1 was seven times greater than that of excretion nutrients, and loading of excretion nutrients onto Nutrient PC2 was nearly four times greater than that of tissue nutrients (Table 5). We find no evidence that tissue nutrient content affected nutrient excretion rates. This result is qualitatively inconsistent with high profile studies of variation in tissue stoichiometry among species, which highlight a negative relationship between tissue and excretion nutrients (Vanni et al. 2002; McIntyre and Flecker 2010), but it is consistent with results from studies within species, which show little correlation between tissue nutrients and excretion nutrients (Higgins et al. 2006; Torres and Vanni 2007).

Excretion and tissue nutrients may be decoupled by the different timescales over which their ecological influences operate. The close relationship between fish excretion and feeding behavior makes excretion rate highly responsive to short-term environmental factors that affect feeding behavior, such as diurnal variation in the strength of predation risk. In contrast, fish tissue nutrients reflect stores of macronutrients like C-rich lipids, N-rich proteins, and P-rich skeletal tissue (the dominant pool of P in adult fish (Hendrixson et al. 2007)) that change with feeding shifts over weeks or even months (Logan et al. 2006; Trudel et al. 2011). As such, we suggest that the independence of excretion and tissue nutrients reflects the largely independent

influences of a top-down driver of short-term behavior (predation risk) and a bottom-up determinant of fish nutritional content (resource availability). Such decoupling of drivers may underlie the lack of strong empirical correlations between tissue and excretion stoichiometry in intraspecific surveys of fish.

Factors structuring Trinidadian stream food webs

The story of life history variation in Trinidadian guppies has emphasized top-down forces as causes of variation in guppy life history traits (Reznick 1983). Across Trinidad, however, gradients in predation risk are mapped onto co-occurring gradients in bottom-up forcing. Streams with predators are always further downstream and tend to have more open canopy and greater availability of limiting light, leading to more resources and faster guppy growth rates (Grether et al. 2001). Indeed, we observed higher guppy condition, as evidenced by higher tissue C and N, in high resource sites. A recent mesocosm experiment by El-Sabaawi et al (2015a) replicated this result by experimentally manipulating canopy cover, leading to greater resource abundance and guppy growth in streams provided with more light. Taken together, these results demonstrate that the availability of light is a powerful force affecting the ecological dynamics and guppy phenotypes of Trinidadian streams.

Both bottom-up and top-down forces influenced resource abundance in this survey. Sites that had more light, as evidenced by more open canopy, had higher stocks of epilithon chlorophyll *a* (Supplemental Table 2). This bottom-up influence was manifest in Resource PC1, which was positively correlated with canopy cover in sites without predators. The presence of predators, however, also influenced food web structure by increasing the abundance of invertebrates (Supplemental Table 5), possibly by reducing the abundance of predators of invertebrates. This influence of predators on resource abundance is apparent in the higher

Resource PC 1 scores of all sites with predators (Figure 1C), regardless of canopy cover. Because both light-driven epilithon chlorophyll and predator-mediated invertebrate abundance load positively on Resource PC1, it is not surprising that the best model for this axis of resource environment included effects of both top-down and bottom-up drivers.

The composition of available resources (*i.e.*, epilithon vs. invertebrates) was captured by a second axis of resource availability (Resource PC2; Figure 1A and 1B). This axis, however, was not consistently explained by either top-down or bottom-up influences acting alone, as indicated by the significant predation × river interaction effect in the best model for Resource PC2. We suggest that variation among rivers is related to other factors unmeasured in this experiment, including the *strength* of predation risk at each site.

Ecological factors beyond canopy cover and predator presence have been shown to mediate ecological dynamics in Trinidadian streams. For instance, disease and the severity of annual floods (Fitzpatrick et al. 2014) influence the population biology, resource environment, and guppy life history of Trinidadian streams. Predation environment, moreover, is much more complex than the binary assessment employed in this study (*i.e.*, with or without predators). Indeed, predation risk occurs as a gradient of intensity that can be an important determinant of the evolutionary divergence among guppy populations (Torres Dowdall et al. 2012).

We did not quantify the strength of predation risk at each pool, but we noted that *Crenicichla* were much more abundant in the Aripo River than they were in the Guanapo. Thus, increased *Crenicichla* abundance in the Aripo may functionally make these sites higher in predation risk than equivalent sites in the Guanapo. Similarly, we noted minor guppy predators, like the catfish *Rhamdia* sp., in *Crenicichla*-free sites in the Guanapo, making these no-*Crenicichla* sites functionally higher in predation risk than equivalent sites in the Aripo. The

largely nocturnal predator *Hoplias* has also been noted in relatively upstream locations within the Guanapo drainage (Gilliam et al. 1993). As a result, the gradient in predation risk was stronger in the Aripo than it was in the Guanapo. This variation in the strength of the gradient in predation risk may underlie some of the predation × river interaction effects that we noted in this study.

Conclusions

Guppy tissue and excretion nutrients were decoupled by the different timescales of the primary drivers of ecological variation in these traits. Tissue nutrients reflected consumption patterns averaged over long timescales, leading to a strong bottom-up signal on tissue C, N and P. In contrast, guppy excretion reflected short-term behavioral responses to predation risk, leading to a strong top-down signal on excretion rate. Predators likely suppress feeding by guppies during certain periods, but, in doing so, predators increase the per capita resource availability and the strength of bottom-up forcing on tissue nutrients. As such, predators are central to understanding variation in tissue and excretion stoichiometry of guppies, but predators have rarely been considered in any analyses of fish stoichiometry. We suggest that our results indicate the necessity of assessing predation risk in attempting to understand or explain intraspecific variation in nutrient excretion.

ACKNOWLEDGEMENTS

This research was funded by an NSF FIBR Grant (DEB 0623632), a Cornell Biogeochemistry and Environmental Biocomplexity Small Grant, a Cornell Andrew W. Mellon Foundation Research Grant, and the Cornell Sigma Xi Society. We thank FIBR Interns, including Connor Fitzpatrick, for assistance with field logistics and sampling. We are grateful to Ronnie Hernandez for maintaining living and research facilities in Trinidad. Comments from Nelson G. Hairston, Jr. and Amy R. McCune substantially improved this manuscript. We thank

Maíra Moraes, Camille Bell and Winnie Chu for assistance identifying and counting invertebrates. Christopher Dalton was supported by an NSF Pre-doctoral Fellowship.

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TABLES

Table 1: Summary of the location, abiotic environment, and guppy resources at all sites surveyed in this study. Sites are ordered in each stream from 1-6, representing from the least to the most complex predator community.

River	Site	Coordinates	*Crenicichla* Status	Temp (C)	Discharge ($L\ s^{-1}$)	Open Canopy (%)	Chl *a* ($\mu g\ cm^{-2}$)	Epilthon AFDM ($\mu g\ cm^{-2}$)	BOM ($\mu g\ cm^{-2}$)	Invert, Density (no. cm^{-2})	Ambient NH_4^+ ($\mu g\ L^{-1}$)	Ambient SRP ($\mu g\ L^{-1}$)
Aripo	1	10°41'24.0", -61°14'13.2"	Absent	24.6	4.3	6	1.23	2337	4619	0.45	3.6	7.7
	2	10°41'13.2", -61°13'58.8"	Absent	25.6	3.4	47	4.12	1327	2459	1.07	3.9	6.9
	3	10°40'44.4", -61°13'40.8"	Absent	26.9	17.8	14	3.94	1243	3140	0.96	7.2	2.6
	4	10°40'12.0", -61°13'40.8"	Present	24.2	97.5	27	2.50	613	1657	2.85	35.9	98.4
	5	10°39'57.6", -61°13'37.2"	Present	24.3	424.5	24	2.39	471	1243	1.88	14.4	26.2
	6	10°39'36.0", -61°13'30.0"	Present	25.0	48.5	33	3.71	941	1231	4.67	11.9	24.3
Guanapo	1	10°42'39.6", -61°15'50.4"	Absent	22.9	7.5	8	0.38	1158	1674	0.42	2.4	27.6
	2	10°42'50.4", -61°16'01.2"	Absent	23.0	102.8	23	1.54	1683	2017	1.08	3.2	17.3
	3	10°42'10.8", -61°15'54.0"	Absent	23.6	194.3	29	0.95	369	1333	0.99	2.7	20.0
	4	10°41'45.6", -61°15'46.8"	Absent	23.8	352.3	28	1.00	207	1283	1.50	4.7	19.0
	5	10°39'32.4", -61°15'57.6"	Present	24.3	0.3	18	1.74	1281	2148	3.18	2.2	3.7
	6	10°39'32.4", -61°15'07.2"	Present	25.1	133.1	53	2.45	555	1924	1.59	9.9	9.9

Table 2: Summary of first two principal components from PC analysis of four resource stocks. PC1 and PC2 explain comparable variation in the resource environment and, together, comprise the majority of variation in resources across the 36 sites in this study.

	PC1	PC2
Percent of variance	39.5	32.9
Cumulative percent of variance	39.5	72.4
Chlorophyll *a* loading	0.49	-0.50
Epilithon loading	0.14	-0.76
Detritus loading	0.62	0.20
Invertebrate loading	0.60	0.37

Table 3: The best supported models for resource PC1 (A.) and PC2 (B.) based on the measurement of chlorophyll *a*, epilithon biomass, detrital matter, and invertebrate density in each of 36 sampled pools in two streams in Trinidad (see Figure 1, Table 1, and Supplemental Materials, Tables 2-5). Models included bottom-up effects (canopy cover, "C"), top-down effects (presence of *Crenicichla*, "P"), river (Guanapo or Aripo, "R"), and all possible two-way interactions among these terms. The term w_i, or the Akaike weight, is the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models include a random effect to account for non-independence of pools clustered around each of six widely spaced access points at each river.

A. Models for Resource PC1	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
P+C+C:P	108.0	0.0	0.27	1.0
C+P	108.9	0.9	0.18	1.6
P	109.3	1.2	0.15	1.9
C+R+P+C:P	110.1	2.1	0.10	2.8
C+R+P	110.8	2.7	0.07	3.9
R+P	111.1	3.1	0.06	4.7
P+C+C:P	108.0	0.0	0.27	1.0
C+P	108.9	0.9	0.18	1.6

B. Models for Resource PC2	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
P+R+R:P	107.2	0.0	0.35	1.0
P	108.4	1.1	0.20	1.7
R+P	109.7	2.5	0.10	3.5
No fixed effect	110.3	3.0	0.08	4.5
C+R+P+R:P	110.4	3.2	0.07	4.9
C+P	110.9	3.7	0.06	6.3
R	112.6	5.3	0.02	14.4
C+R+P	112.6	5.3	0.02	14.4

Table 4: Average diet composition of guppies at each of twelve sampling sites. Averages represent mean of five guppies collected from each of three pools (for 15 total guppies) at each site. Site numbers (1-6) are as in Table 1. Values in table represent the area of the slide (in mm^2) occupied by each diet type.

River	Site	Invert Area	Detritus Area	Fil. Algae Area	Diatom Area	Empty Area
Aripo	1	2.52	27.70	0.00	0.00	12.13
	2	1.73	27.65	0.06	0.00	16.67
	3	3.74	34.02	0.00	0.00	11.00
	4	9.19	5.76	0.00	0.00	27.47
	5	5.50	8.22	0.00	0.00	18.80
	6	9.23	6.13	0.00	0.00	25.20
Guanapo	1	5.95	14.33	0.00	0.00	17.80
	2	1.89	32.52	0.00	0.10	4.93
	3	7.67	23.76	0.00	0.12	9.14
	4	5.32	18.95	0.00	0.26	11.67
	5	1.62	19.47	0.00	0.04	9.90
	6	5.04	22.87	0.01	0.29	13.40

Table5: Summary of first two principal components from PC analysis of three diet items. PC1 and PC2 comprise the vast majority of variation in diet among 159 guppies assessed for dietary content.

	PC1	PC2
Percent of variance	60.0	28.3
Cumulative percent of variance	60.0	88.3
Diet invertebrate loading	0.43	0.87
Diet detritus loading	-0.66	0.12
Empty gut space loading	0.61	-0.48

Table 6: The best supported models for diet PC1 (A.) and PC2 (B.) based on the measurement of dietary invertebrates, detritus, and empty space in 159 guppies from 36 sampled pools in two streams in Trinidad. Models included bottom up effects (resource PC1 and PC2; "RPC1" and "RPC2"), top down effects (presence of *Crenicichla*, "P"), river (Guanapo or Aripo, "R"), and all possible two-way interactions among these terms. The term w_i, or the Akaike weight, is the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models include a random effect to account for non-independence of pools clustered around each of six widely spaced access points at each river.

A. Models for Diet PC1	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
RPC2+R+P+R:P	511.2	0.0	0.33	1.0
RPC2+R+P+RPC2:R	512.4	1.2	0.18	1.8
RPC2+R+RPC2:R	512.9	1.6	0.15	2.2
P+R+R:P	513.0	1.8	0.14	2.4
RPC1+R+P+R:P	514.7	3.4	0.06	5.6
RPC2+R+P	516.0	4.7	0.03	10.6
RPC2+P	516.3	5.0	0.03	12.3

B. Models for Diet PC2	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
RPC2+R	429.9	0.0	0.13	1.0
RPC2	430.1	0.2	0.11	1.1
RPC2+R+P+RPC2:P	430.2	0.3	0.11	1.1
RPC2+P	431.4	1.5	0.06	2.1
R	431.7	1.8	0.05	2.4
RPC2+R+P	431.9	2.0	0.05	2.7
1	432.0	2.1	0.04	2.9

Table 7: Average tissue and excretion nutrient composition of guppies at each of twelve sampling sites. Averages represent mean of ten female guppies collected from one pool at each site. Site numbers (1-6) are as in Table 1. Values in table represent percent of dry weight composed of each element and the size-corrected N and P excretion (as in Torres and Vanni 2007). Size corrected variables are calculated by dividing the measured excretion by the slope of the global relationship between fish wet weight and measured excretion rate in this dataset, so units are as follows: N excretion: $\mu g\ N\ h^{-1}\ g^{-0.46}$; P excretion: $\mu g\ P\ h^{-1}\ g^{-0.41}$.

River	Site	% C	%N	%P	N Exc	P Exc
Aripo	1	38.86	9.14	3.97	18.03	1.59
	2	42.47	8.98	3.59	36.13	0.78
	3	39.91	9.05	3.72	40.65	1.60
	4	42.34	9.46	3.64	26.56	0.65
	5	41.57	9.33	3.43	21.28	0.42
	6	42.10	9.59	3.48	17.01	0.25
Guanapo	1	43.89	9.65	3.06	11.95	0.71
	2	39.95	9.22	3.78	21.80	0.56
	3	43.98	9.60	2.72	41.67	0.38
	4	44.31	9.61	2.63	45.16	1.69
	5	41.21	9.58	3.25	21.93	0.33
	6	45.39	9.60	2.15	20.36	0.11

Table 8: Summary of first two principal components from PC analysis of three measures of tissue nutrients and two measures of excreted nutrients. PC1 and PC2 comprise the majority of variation in tissue and excretion stoichiometry among 102 guppies from twelve different access points in each of two rivers.

	PC1	PC2
Percent of variance	46.2	30.0
Cumulative percent of variance	46.2	76.2
Tissue Percent C loading	0.59	-0.15
Tissue Percent N loading	0.50	-0.01
Tissue Percent P loading	-0.57	0.21
Excretion N loading	-0.05	-0.73
Excretion P loading	-0.27	-0.63

Table 9: The best supported models for Nutrient PC1 (A.) and PC2 (B.) based on the measurement of tissue C, N and P and excretion N and P from 102 guppies from 12 sampled pools in two streams in Trinidad. Models included bottom up effects (diet PC1 and PC2; "DPC1" and "DPC2"), top down effects (presence of *Crenicichla*, "P"), river (Guanapo or Aripo, "R"), and all possible two-way interactions among these terms. The term w_i, or the Akaike weight, is the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models include a random effect to account for non-independence of guppies collected at the same pool.

A. Models for Nutrient PC1	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
DPC1+R+DPC1:R	309.3	0.0	0.18	1.0
DPC1+R+P+DPC1:R	309.3	0.0	0.18	1.0
DPC1+R	309.9	0.6	0.13	1.4
DPC1+R+P+R:P	310.8	1.6	0.08	2.2
DPC1+R+P+DPC1:P	311.4	2.1	0.06	2.9
DPC1+R+P+DPC1:R+P:R	311.6	2.3	0.06	3.2
DPC1+R+P	311.9	2.6	0.05	3.7

B. Models for Nutrient PC2	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
P	312.6	0.0	0.19	1.0
DPC2 + P	313.7	1.0	0.11	1.7
No Fixed Effects	314.6	1.9	0.07	2.6
DPC1 + P	314.7	2.1	0.07	2.9
DPC1 + P	314.7	2.1	0.07	2.9
R + P	314.8	2.1	0.06	2.9
DPC2	315.4	2.8	0.05	4.0

FIGURES

Figure 1: Resource environment of 36 pools across 2 streams, Aripo (blue circles) and Guanapo (red triangles). **A and B:** Plot of PC1 and PC2 for Aripo only (**A.**) and Guanapo only (**B.**) with symbols colored according to the presence of *Crenicichla* (light) or absence of *Crenicichla* (dark). Light gray, thin arrows portray loading of each of the four resources used to conduct principle components analysis: chlorophyll *a* concentration (Chl a), epilithon biomass (Epi Biomass), detrital biomass (BOM), and benthic invertebrate density (Inverts). All four variables load positively on PC1, which describes sites with higher quantities of all resource stocks, and invertebrates and detritus load positively on PC2 while epilithon biomass and chlorophyll content load negatively on this axis. Thicker blue and red arrows represent the average of all sites with *Crenicichla* (light arrows) and without *Crenicichla* (dark arrows). **C. and D.**: Illustrations of best fit models (see results) for Resource PC1 and PC2. **C.** Resource PC1 scores scale positively with the amount of open canopy in sites without predators (dark points and lines), and sites with predators (light points and lines) have higher resource PC1 scores, but only at low light levels. Sites with more light, and with predators, thus have higher stocks of resources. **D.** Resource PC2 scores were only affected by a river × predation interaction, as sites without *Crenicichla* in the Aripo had substantially lower resource PC2 scores, indicating relatively high quantities of epilithon and low quantities of invertebrates. Dashed lines represent standard errors of model fits.

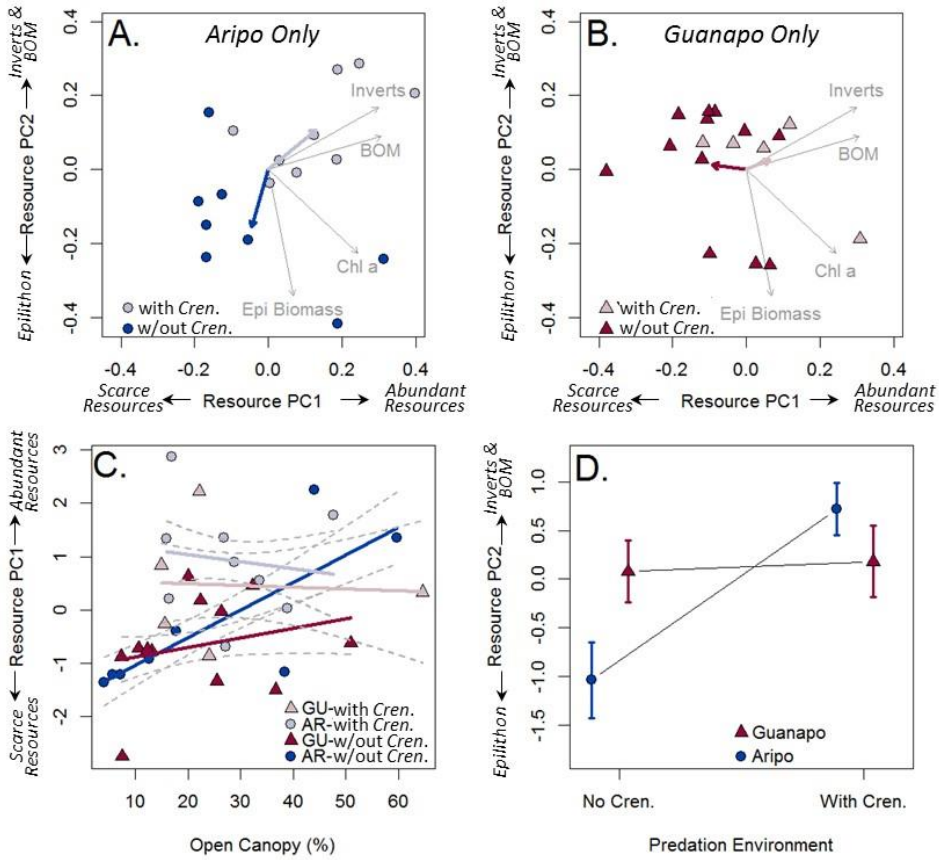


Figure 2: Diets of 159 guppies from 36 pools across 2 streams, Aripo (blue circles) and Guanapo (red triangles). **A and B:** Plot of PC1 and PC2 for Aripo only (**A.**) and Guanapo only (**B.**) with symbols colored according to the presence of *Crenicichla* (light) or absence of *Crenicichla* (dark). Light gray, thin arrows portray loading of each of the three diet items used to conduct principle components analysis: invertebrates, detritus, and unfilled space. Invertebrates load positively on both PC1 and PC2, Detritus loads negatively and primarily on PC1, and Empty space loads positively on PC1 and negatively on PC2. Thicker blue and red arrows represent the average of all sites with *Crenicichla* (light arrows) and without *Crenicichla* (dark arrows). **C. and D.**: Illustrations of best fit models (see results) for Diet PC1 and PC2. **C.** Diet PC1 scores scale positively with Resource PC2 (differentiating sites with lots of invertebrates from sites with abundant epilithon; Figure 1) and are elevated in Aripo HP sites (which also have very high Resource PC2 scores). **D.** Diet PC2 scales positively with Resource PC2. The abundance of invertebrates relative to epilithon thus appears to control whether guppies consume detritus (less detritus consumption when invertebrates are abundant) and how much empty space guppy guts have (less empty space when invertebrates are abundant). **hh**

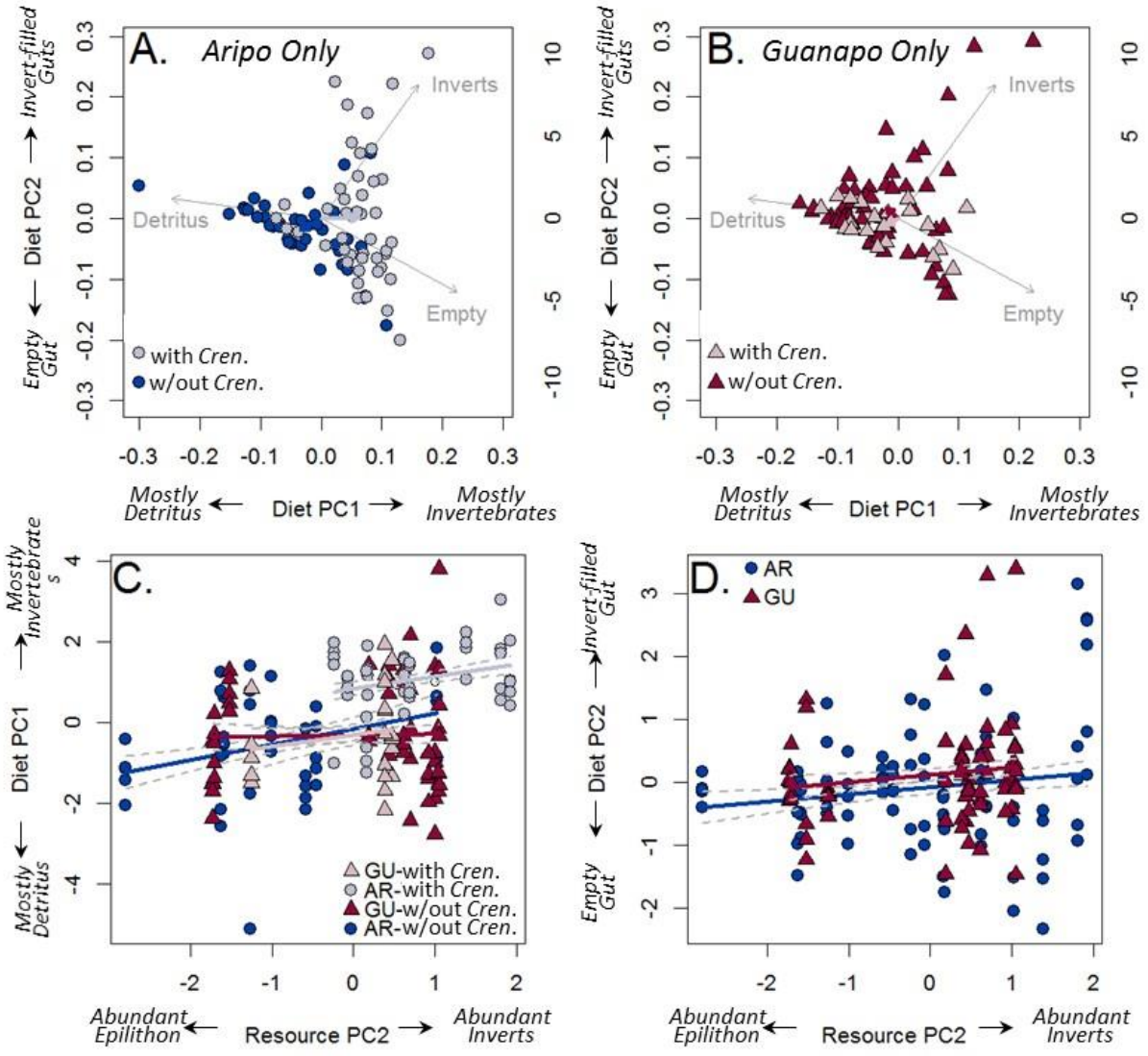
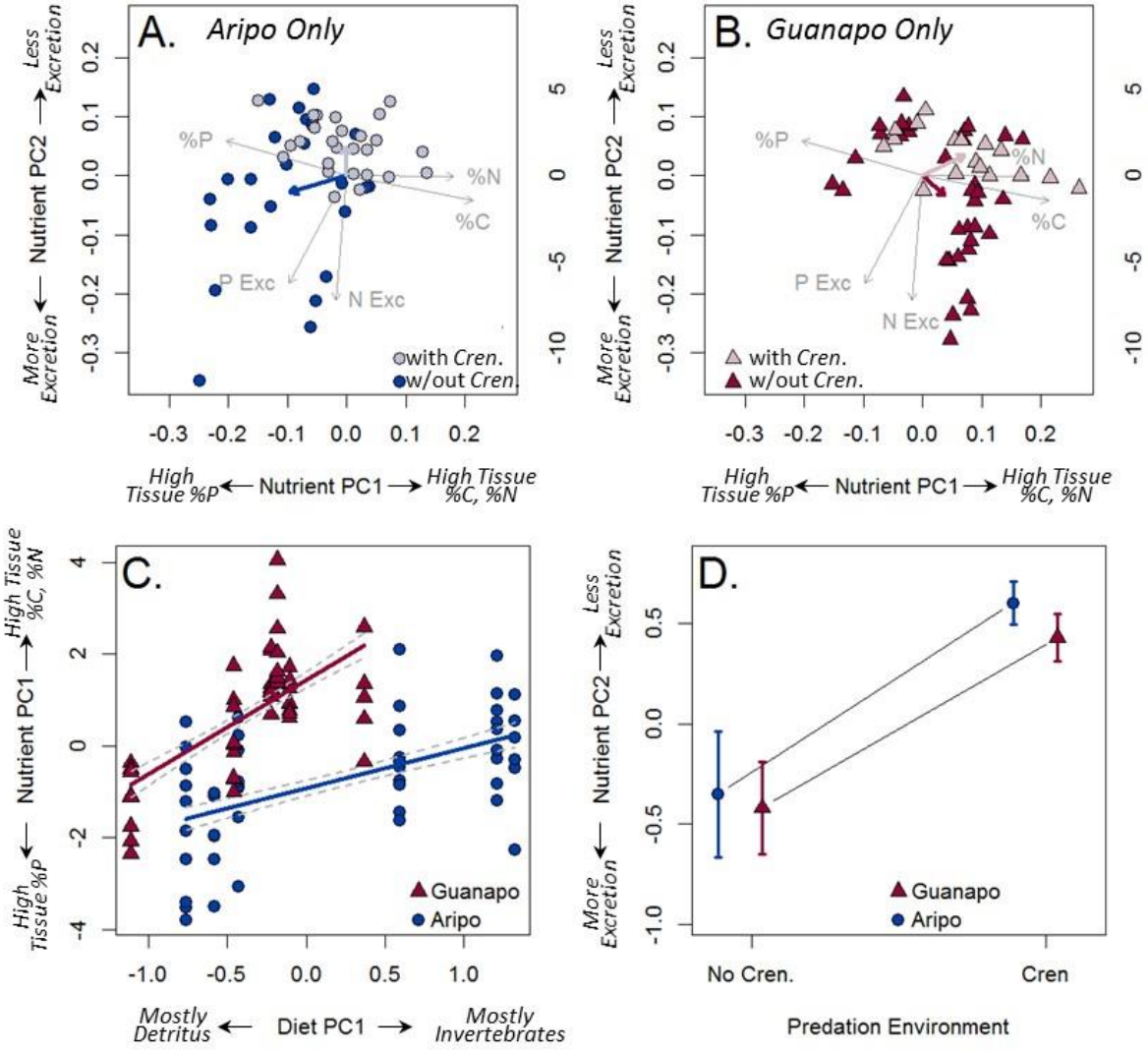


Figure 3: Tissue and excretion nutrients of 102 guppies from 12 pools across 2 streams, Aripo (blue circles) and Guanapo (red triangles). **A and B:** Plot of PC1 and PC2 for Aripo only (**A.**) and Guanapo only (**B.**) with symbols colored according to the presence of *Crenicichla* (light) or absence of *Crenicichla* (dark). Light gray, thin arrows portray loading of each of the five measures of tissue and excretion nutrients: tissue percent C, N, and P and excretion N and P. Tissue C and N load positively and tissue P negatively on both PC1, and excretion loading on PC1 is limited. N and P excretion load negatively on PC2, and tissue loading on PC2 is minimal. Thicker blue and red arrows represent the average of all sites with *Crenicichla* (light arrows) and without *Crenicichla* (dark arrows). **C. and D.**: Illustrations of best fit models (see results) for Guppy Nutrient PC1 and PC2. **C.** Guppy Nutrient PC1 scales positively with Diet PC1 (differentiating guppies with invertebrate diets from guppies with detrital diets), especially in the Guanapo. **D.** Nutrient PC2 is higher in sites with *Crenicichla*, indicating less excretion in these sites. Guppy tissue nutrients thus relate to diet, with C and N increasing and P decreasing on higher quality diets, and guppy excretion relates best to predation environment, with at risk guppies excreting less.



CHAPTER THREE: METABOLIC STOICHIOMETRY AND THE ECOLOGY OF FEAR IN TRINIDADIAN GUPPIES: CONSEQUENCES FOR LIFE HISTORIES AND STREAM ECOSYSTEMS

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ABSTRACT

Consumer-driven nutrient recycling, the release of chemicals as byproducts and excesses of consumer physiology, can alter ecosystems by changing the availability of limiting nutrients at the base of the food web. The mere presence of predators can alter consumer physiology by restricting food intake and inducing stress. Predation risk, then, can influence ecosystem function by modifying the role of prey as nutrient recyclers, yet there are few empirical tests of how predation risk alters nutrient recycling by prey. Here, we present the results of a test for the effects of predation risk on the carbon (C) and nitrogen (N) budgets of Trinidadian guppies (*Poecilia reticulata*). We reared female guppies for seven weeks on diets of varying quality, and we compared control individuals to those exposed continuously to chemical cues emitted by a guppy predator, *Crenicichla frenata*. We measured food consumption, growth rate, tissue elemental stoichiometry and N excretion by guppies on all treatments. Guppies strongly reduced food intake in the presence of predator cues; however, cue-exposed guppies assimilated nutrients more efficiently than did controls. Specifically, cue-exposed guppies strongly increased N retention efficiency while only moderately increasing C efficiency. Consequently, guppies reared with predator cues excreted 39% less nitrogen than control guppies. We suggest that reduced foraging, enhanced nutrient efficiency, and decreased N excretion are adaptive responses to the extrinsic mortality threat posed by guppy predators. The resulting substantial reduction in N excretion by guppies may influence ecosystem function in natural streams by reducing the supply of a limiting nutrient.

Keywords: general stress, non-consumptive predator effect, behaviorally-mediated trophic cascade, nutrient excretion

INTRODUCTION

Animal excretion and egestion shape ecological dynamics in nearly every ecosystem– from coral reefs to boreal forests (Hemminga 1991; Hobbs 1996; Vanni 2002; Nichols et al. 2008; Layman et al. 2013; Murray et al. 2013). The ubiquity of animal-mediated nutrient cycling effects has flipped our understanding of how consumers affect ecosystems on its head. Animals control ecosystems not just from the top-down, through consumption, but also from the bottom-up, through release of limiting nutrients as byproducts of metabolism (Vanni and Layne 1997; Knoll et al. 2009). Describing the drivers and constraints on nutrient recycling by animals is thus central to understanding ecological dynamics (Elser and Urabe 1999).

Predators represent a potentially important but scarcely understood influence on nutrient recycling. Predators have traditionally been thought to affect communities and ecosystems through consumption. Indeed, predators alter the abundance of prey, with cascading effects on lower trophic levels that are standard fodder of introductory ecology (Hairston et al. 1960; Carpenter et al. 1985). Predation risk also alters the physiology (Stoks et al. 2005; Barry and Syal 2012; Clinchy et al. 2013; Handelsman et al. 2013), behavior (Peckarsky 1980; Beckerman et al. 1997; Hebblewhite and Merrill 2009), morphology (Stemberger and Gilbert 1987; Tollrian 1995; Lardner 2000), and life history of prey (Sih and Moore 1993; Warkentin 1995; Kusch and Chivers 2004), influencing ecological dynamics by changing the effectiveness of prey as consumers of, and competitors for, resources (Schmitz et al. 2004). These same physiological, behavioral, morphological and life history traits are also closely linked to rates of consumer nutrient recycling (McIntyre and Flecker 2010), yet the influence of predation risk as a control on nutrient recycling by prey has only recently received empirical attention.

A set of recent papers has presented the 'proof-of-concept' that predators affect ecosystems by changing the nutrients recycled by their prey (Hawlena and Schmitz 2010a; Hawlena and Schmitz 2010b; Hawlena et al. 2012; Leroux et al. 2012). The mere presence of predatory spiders induces altered metabolism, food selectivity, digestive efficiency, and tissue nutrient content of grasshoppers (Hawlena and Schmitz 2010a). The changed metabolism and nutrient recycling of predator-exposed grasshoppers then alters the decomposition and respiration of soils incubated with predator-exposed grasshoppers (Hawlena et al. 2012; Leroux et al. 2012). These results indicate that predators can alter nutrient recycling by prey and measurably impact ecosystem function, and here we seek to build on these results by assessing the role of predation risk in the nutrient budgets of another model of predator-prey interactions: the Trinidadian guppy (*Poecilia reticulata*).

In Trinidad, guppies exist in a mosaic of predation risk that influences their trait expression through evolution and phenotypic plasticity. While some stream segments that hold populations of guppies completely lack effective piscivores, guppy predators are abundant and diverse in many others (Gilliam et al. 1993). Guppy behaviors change in the presence of predators, as guppies spend less time feeding when predators are present (Fraser and Gilliam 1987; Fraser and Gilliam 1992; Fraser et al. 2004), and guppies at sites with predators feed more selectively on food items that more closely match their own tissue nutrient content (Zandonà et al. 2011). Predation risk induces plastic change in guppy life history traits (*e.g.*, growth rate and size at maturity; Torres-Dowdall et al. 2012; Handelsman et al. 2013), and some populations (notably those from low predation environments) show predator-induced plasticity in physiological traits such as glucocorticoid expression and respiration rate (Handelsman et al. 2013; Fischer et al. 2014). Experimental introductions of guppies to previously uncolonized,

low-predation risk environments have demonstrated that guppies rapidly evolve altered life history traits within only a few years in these natural low-predation environments (Reznick and Bryga 1987; Reznick et al. 1997). Guppies have thus been a model for understanding predator-driven trait change via evolutionary and ecological mechanisms.

Here, we present the results of a laboratory growth experiment designed to assess the influence of predation risk on nutrient recycling by guppies. We reared blocks of full sibling guppies for seven weeks either in the presence or absence of predator cues. Within each block, we crossed our predation risk treatment with two diet quality treatments: guppies were reared on either a high or a low carbon:nitrogen (C:N) diet. Over the entire growth period, we assessed the influence of diet and predation risk on the amount of food consumed, the amount of growth accrued, and the efficiency of the conversion of nutrients in food to nutrients in tissue. We then measured nitrogen (N) excretion to assess the influence of predator-induced behavioral and metabolic changes on guppy-driven nutrient recycling.

METHODS

Experimental Subjects

We collected *P. reticulata* from the Aripo drainage of the Northern Range Mountains of Trinidad in April 2011. We collected fish from a high-predation locality where guppies coexist with a diversity of piscivorous fish (high predation, HP fish). HP fish were collected from a several-hundred meter stream reach in the Aripo River (GPS: 10.66568N 61.22789W). Wild-caught and first-generation lab fish were kept individually in 3-L tanks in a recirculating system (Aquatic Habitats, Apopka, FL; 12L:12D, temperature 25 ± 1°C) and fed twice daily (AM: Tetramin^{TM} tropical fish flake paste, PM: hatched Artemia cysts). We propagated two lab-born

generations from wild collected Aripo females. Family lines were generated by randomly crossing lab-born fish within each generation, as has previously been conducted for other studies of second generation lab bred guppies. Lab selection effects were minimized by collecting a large sample of guppies from the wild (>100), minimizing mortality in the lab and propagating each lab generation from a large subsample of females from the previous generation (48 pairs from the original 100 collected fish).

Second lab-born generations of Aripo HP guppies (F2) were removed from breeding tanks and reared in 1.5-L tanks until individuals could be reliably sexed (>25 days post parturition). The feeding ration was the same as the above, but these juvenile guppies were fed a base diet of Brine Shrimp Flake (Brine Shrimp Direct, Ogden, UT) with fish oil added (Menhaden oil, Dyets, Inc., Bethlehem, PA) to increase lipid content from 10% to 15%. Previous studies on guppy physiology have used fish oil as an important source of lipids (Shim and Ho 1989). We reared each brood from a single male-female pairing in a single 1.5-L tank on an ad-lib feeding regimen until beginning the experiment. At the initiation of the experiment, virgin female guppies from each brood were anesthetized with MS-222. We verified the sex of each fish under a dissecting microscope and measured each for length and weight. Females were then randomly assigned to one of four treatments (see below).

Experimental Design

The experiment was a factorial design with two main effects: diet quality (low vs. high C:N) and predation risk (with vs. without predator cue). These four treatments were blocked spatially on a single vertical shelving unit, with each block containing only full siblings from the same brood, such that brood effects could not be distinguished from spatial blocking effects. We

established seventeen blocks per treatment, though one block had a fish escape from its tank in the second week of the experiment and was excluded from the analysis. The combined effect of brood and block is henceforth referred to as "block" effects, though this variation includes both the effect of the common rearing and genetic background of the brood and any consistent variation in the rearing environment due to the location within the shelving unit.

The diet treatment was created by altering a base diet of Brine Shrimp Flake (Brine Shrimp Direct, Ogden, UT) by adding fish oil (Menhaden oil, Dyets, Inc., Bethlehem, PA) to increase lipid content from 10% to 20%. The effect of this diet addition was to increase the diet's carbon (C) content, decrease its N content, and increase its C:N (Table 1). We will refer to these diets as *High C:N* and *Low C:N*, yet the high C:N diet will also be higher in C and lipid and lower in N and protein content than the low C:N diet. Causally, we cannot distinguish the effect of C:N, *per se*, from the effects of elevated C or lipid, decreased nitrogen or protein, or altered lipid:protein ratio. Diets were ground to a homogenous powder using a mortar and pestle and stored at -20 °C. Immediately prior to each feeding, we warmed 1-2 g of each dry diet to room temperature and mixed the dry food with water at a 2:3 food:water ratio, such that the diet was 40% ± 0.2% (standard error of 20 replicate samples) dry weight. Each paste-like diet was then loaded into a luer tip micro-syringe (Hamilton, Reno, NV), and a known volume of the appropriate treatment food was delivered to each guppy. Guppies were always fed *ad libitum*, as some leftover food was collected from every tank at every feeding. Guppies were fed two times per day as in studies of guppy life history and physiological responses to predation risk (Torres-Dowdall et al. 2012; Handelsman et al. 2013).

The predation cue treatment was introduced by the water plumbed through each guppy tank on a recirculating rack. Each 1.5-L guppy tank either received flow from a 120-L supply

tank housing a single pike cichlid (*Crenicichla frenata)* or a 120-L supply tank with no fish in it. *Crenicichla* is a dominant guppy predator, and previous studies have shown guppies respond strongly to cues released by this predator (Torres-Dowdall et al. 2012; Handelsman et al. 2013). Our design was comparable to the design used in these studies, except that the *Crenicichla* in our experiment was reared on frozen brine shrimp. Guppies thus only received predator kairomones and no conspecific alarm cues. We changed one half of the volume of each supply tank daily and replaced it with water from a predator-free source tank that contained deionized water, instant ocean and sodium bicarbonate (Instant Ocean: 0.88 $g \cdot L^{-1}$; Sodium Bicarbonate: 0.14 $g \cdot L^{-1}$).

In this experiment, we reared guppies in individual tanks, largely because group rearing of guppies creates substantial variation in physiological and life history variables related to dominance hierarchies established by guppies reared in groups in lab conditions (Sullam *et al.*, in revision). One prominent response of guppies to predation risk, however, is an increase in shoaling (unpublished data, Weetman et al. 1999; Song et al. 2011). Shoaling can alter the behaviors of fish and likely alters the physiology of stress (Rehnberg and Smith 1988). It is possible that allowing guppies to shoal would have changed the behavioral and physiological response that we observed in this study. To test this hypothesis, we conducted a pilot study comparing the excretion rates of predator exposed and predator naïve guppies reared in tanks individually or in small groups (n=3 per tank). We found no effect of group size on the growth or excretion rates of guppies reared in the presence or absence of predator cues, but sample sizes were small on all treatments (n = 4 blocks). These results suggest that the physiological response of guppies to predation risk would not be substantially altered by group size; however, more rigorous testing with better replication would be necessary to truly test this intriguing hypothesis.

Response Variables

We assayed guppies for standard length and weight at 17, 34, and 45 days after the initiation of the experiment. The duration of the experiment was set to roughly match the onset of first parturition in mated female guppies and to thus reflect the investment in a single brood of eggs. On days 17 and 34, we anesthetized guppies with MS-222, measured standard length with digital calipers to the nearest 0.01 mm and weighed to the nearest mg. On day 45, guppies were euthanized with an overdose of MS-222, photographed using a handheld digital camera with a metric scale bar to estimate standard length, and measured for wet weight. We dissected and photographed the digestive tract and reproductive tissues of each guppy and measured for wet weight. All tissues were dried to constant mass at 55°C and reweighed to estimate total dry mass.

We estimated food consumption by collecting uneaten food after each feeding using a modified plastic pipette. Food was collected at least 90 minutes after feeding to allow guppies time to finish consumption. Uneaten food and water incidentally acquired during food collection were placed on pre-weighed metal drying pans labeled for each fish and dried at 55 °C between collections. The total dry weight of collected food was measured weekly, by allowing pans to dry to constant mass. Drying dishes were then ashed for 4 hours at 500°C and reweighed to estimate the ash-free dry mass (AFDM) of uneaten food collected. The loss of nutrient from the diet to leaching was corrected using a set of 12 fishless 1.5-L tanks that were plumbed, fed and food collected as if they contained fish. AFDM loss in these tanks was assumed to be due to leaching, and the recovered food from all other tanks was multiplied by the ratio of original food to recovered food from these tanks, thereby estimating the mass of food that would have remained if there was no leaching.

To estimate guppy tissue C and N content, guppy somatic and reproductive tissues were ground into a homogeneous powder using a Wig-L Bug®. Subsamples (2 mg for C and N) of

reproductive and somatic tissue from each guppy as well as replicates of each diet were then assayed for C and N content following methods described in El-Sabaawi et al. (2012). Briefly, ~2 mg subsamples of homogenized guppy tissue or fish diet were weighed to the nearest thousandth of a mg, and the percent C and N of each sample was assayed on a CNH analyzer (Vario EL III elemental analyzer, Elementar, Hanau Germany).

At the conclusion of the experiment, each fish was assayed for NH_4^+ excretion both in the absorptive and post-absorptive states. Within 60 minutes of food delivery, we removed guppies from their experimental tanks, introduced them into plastic beakers containing 500 mL of UV-sterilized water from their source tank, and placed each in an opaque shelter to minimize disturbance during the incubation. After a 20-minute acclimation period, we collected subsamples of each beaker using a 60 mL plastic syringe. We collected a second sample from each beaker after another 60 minutes. To estimate excretion in a non-absorptive state, we collected a second set of excretion samples after eight hours, again with a 60-minute incubation. The timing of each sample was dictated by prior pilot research, which showed (i) 20 minutes of acclimation was sufficient to alleviate handling stress effects, (ii) the absorptive peak in NH_4^+ excretion was present for the first four hours after feeding, and (iii) post-absorptive metabolism was reached within six hours of fasting.

Subsamples for NH_4^+ analysis were filtered through an ashed filter (GF/F Whatman), refrigerated within 20 minutes of collection, and analyzed within 12 hours. NH_4^+ concentrations were measured on an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA), equipped with a UV filter (Holmes et al. 1999; Taylor et al. 2007).

Data analysis

Condition factor was defined as the guppy weight divided by the length to the third power, times 100,000 (Bolger and Connolly 1989). Specific growth was calculated as the difference in log-transformed initial and final mass divided by the number of days elapsed between measurements.

We quantified food consumption by comparing uneaten food remaining in each tank to the total food fed to each fish. Starting with the total amount of AFDM recovered from each tank (see above), we multiplied this value by the ratio of dry matter to AFDM in the original food. This calculation estimated the total amount of dry food uneaten by each fish over the experiment. By subtracting this value from the total amount of dry food fed during the experiment, we estimated total dry food consumption. We multiplied this value by the percent of C and N in each diet to estimate total C and N consumption.

C and N use efficiency were calculated by dividing the amount of C and N in guppy tissues after the 45 day experiment by the amount of C and N consumed during the experiment. Because much of the C and N consumed by organisms must be used to repair and maintain metabolically active tissues, this measure provides an index of how much consumed C and N are used for growth, reproduction and maintenance of existing tissues.

We estimated the proportion of consumed N excreted by all guppies in the final week of the experiment. We assumed that the daily N consumption of a guppy on the day that excretion was measured matched the total consumption estimated over the previous seven days (estimated using the methods above) divided by seven days. We used our hourly absorptive and post-absorptive N excretion estimates to approximate total daily N excretion. We used previous data that indicated the absorptive peak in NH_4^+ excretion lasts for four hours after a meal and is

followed by rapid, exponential decay in NH_4^+ excretion to post-absorptive levels by six hours post-feeding. We then estimated total daily NH_4^+ excretion as: (2 meals per day × 4 hrs per meal of absorptive metabolism × each fish's hourly absorptive NH_4^+ excretion) + (16 hours per day of post-absorptive metabolism × each fish's hourly post-absorptive NH_4^+ excretion). We estimated excretory N efficiency as the fraction of consumed N not estimated to have been excreted as NH_4^+ over the course of an entire day.

Statistics

Treatment effects on response variables were assessed using linear mixed models. The most complex, biologically-feasible models were simplified to the best fit model using likelihood-ratio tests and Akaike Information Criteria (AIC) score comparison (Akaike 1974). All models included "block" as a random effect to account for non-independence of individuals from the same brood and the non-independence of blocks which were arrayed spatially within the shelving unit. Treatment effects on length and weight were assessed using a model of log-transformed guppy length and weight with log-transformed guppy age as a covariate and individual guppy ID as a random effect to account for non-independence of multiple measures of the same fish. For all traits that scale allometrically, we used log transformation of both the response variable and covariate. Suitability of data variance distributions to this analytic method was validated using the package gvlma on comparable models without random effects (Pena and Slate 2006) and by visual inspection of plots of variance distributions and residuals. All statistics were performed using R statistical software (R-Team 2010). We interpreted p values < 0.05 as significant and $0.05 – 0.10$ as marginally significant.

We here present results based on AIC-score based model comparison and likelihood ratio tests. The details of the model comparisons and likelihood ratio tests are presented in tables in the Electronic Supplemental materials (Table S1 – S8). Unless otherwise stated, all treatment effects presented below represent statistically significant ($p < 0.05$) likelihood ratio tests of models with the listed terms and models without those individual terms. All treatment effects listed below are also present in the best model for each response variable.

RESULTS

Total Food Consumption

The amounts of dry food and C consumed were affected by only the predator cue treatment (Table S1, Figure 1a, Figure 1b). Cue guppies consumed less dry mass, less C and less N than control guppies. Because the high C:N diet was lower in N content (Table 1), high C:N diet guppies consumed less N despite consuming comparable amounts of C (Table S1b, Figure 1b).

Recycled Wastes - Excretion

Excreted ammonia (log-transformed) both 1 hour and eight hours post-feed (Table S2a, Table S2b; Figure 2a, Figure 2b) was affected by both the diet and cue treatments. In general (and as expected based on metabolic allometry), excretion rates increased at a decreasing rate with fish wet weight (*i.e.*, slope of log-log relationship of excretion with fish weight was less than 1). At any weight and at both time points, N excretion was lower for high C:N diet and with-cue treatments (Table S2a, Table S2b; Figure 2a, Figure 2b). Variation in N excretion eight hours post-feeding was also explained by an interaction between weight and diet, as N excretion

by low C:N diet guppies increased more slowly with weight than that of high C:N diet guppies (Figure 2b, Table S2b).

Variation in modeled total daily N excretion was best explained by a model fully factorial for daily N consumed, diet treatment and cue treatment (Table S2c, Figure 2c). The good fit of this complex model is likely explained by the low slope of the relationship between daily N excretion with daily N consumption for the no-cue high-C:N diet treatment group (Figure 2c). Aside from this three way interaction of daily N consumption × cue treatment × diet treatment, the main effects of diet and cue treatment and daily N consumption all significantly increased the explanatory power of the model (Table S2c). Daily N consumption increased N excretion in all treatments, and, at a given level of N consumption, high C:N diet and with-cue guppies excreted less N than low C:N diet and no-cue guppies.

Growth efficiency

Nutrient use efficiency was estimated as the grams of nutrient retained in tissue divided by the grams of nutrient consumed during the experiment. The nutrient use efficiency for C (NUE_C) was affected by diet and marginally by predator cue (Table S3a, Figure 3a). Guppies reared on the high C:N diet had lower NUE_C, indicating a greater proportion of consumed C was released as waste. Cue-exposed guppies had marginally higher NUE_C. Nitrogen use efficiency (NUE_N) was higher for cue reared guppies than control guppies (Table S3b, Figure 3b), indicating cue-reared guppies released less of consumed N as waste. NUE_N was not affected by diet.

Dividing NUE_C by NUE_N yields $NUE_{C:N}$, the nutrient use efficiency of C relative to that of N (Table S3c, Figure 3c). A quotient of 1 reflects that C and N were retained with equal

efficiency, suggestive of a diet balanced relative to organism demand. Our no-cue, low C:N diet treatment yielded a $NUE_{C:N}$ not substantially different from 1(Figure 3c). The balance of this diet with organism demand likely is related to the high growth performance of this treatment combination. Both the cue and high C:N diet treatments significantly reduced $NUE_{C:N}$ (Table S3c, Figure 3c), indicating that the high C:N and cue treatments reduced the efficiency of C utilization relative to that of N.

Tissue Stoichiometry

The percent of tissue composed of any single element will be altered by changes in the abundance of any other element in tissue (Sterner and Elser 2002). This effect makes interpretation of percent of dry weight metrics difficult, as we cannot discern whether a change in the percent composition of a single element reflects changes in homeostatic regulation of that element or is a byproduct of change in homeostatic regulation of other elements. To assess how our treatments altered the allometry of deposition of C and N into new biomass, we regressed total tissue C and N against standard length, log-transforming both to account for allometry (Torres and Vanni 2007).

Diet did not explain a significant proportion of variation in tissue C or N (Table S4a, Table S4b, Figure 4a, Figure 4b). Cue-reared guppies had less C at a given length than control guppies (Figure 4a, Table S4a), likely due to lower stores of C in lipids. Cue treatment did not affect length-specific tissue N (Table S4b, Figure 4b). Cue and diet affected tissue C:N (Table S4c, Figure 4c). Tissue C:N was best explained by a model with standard length, diet, cue, and an interaction between standard length and cue. Tissue C:N increased with length, but only for

no-cue reared guppies. No-cue reared guppies and guppies reared on the high-C:N diet had higher tissue C:N than cue-reared fish or fish reared on a low C:N diet (Table S4c, Figure 4c).

Length, weight, growth and reproductive allocation

Guppy growth was negatively affected by both predator cue and high C:N diet treatments (Table S5a, Table S5b, Figure S1a, Figure S1b). The increase in length and weight with age was slower for fish reared with the predator cue (Figure S1a, Figure S1b), and guppies reared on the high C:N diet were always smaller than those on the low C:N diet. Condition factor increased with age in the no-cue environment but not in the cue environment (Table S5c, Figure S1c), and it was not different among diets (Table S5c, Figure S1c). Specific growth slowed with age and was lower on the high C:N diet and with-cue treatments (Table S5d). The high C:N diet's negative effect on growth was greater for no-cue guppies (diet × cue interaction), and the decrease in specific growth with age was stronger for no-cue fish (age × cue interaction). The proportional allocation of C, N, and dry weight to reproductive versus somatic tissues was not affected by either treatment (Tables S6 – S8).

DISCUSSION

Predator cues reduced guppy N excretion nearly 40% by reducing guppy size, feeding activity, and protein catabolism. Our estimates of nutrient conversion efficiency indicate that reduced N excretion under predation risk reflects both lower food intake and physiological accommodation of reduced food intake. Here, we consider the implications of these results for N cycling in Trinidadian streams and for the adaptive significance of the physiological responses of prey to predation risk and their implications for the role of prey as nutrient recyclers.

Predation Risk Effects on Nitrogen Excretion

N excretion by fish can strongly influence ecological dynamics (McIntyre et al. 2007; McIntyre et al. 2008). Variation in the N excretion of wild fish has largely been assessed as differences among species (McIntyre and Flecker 2010), but individuals and populations within species can show as much or more variation than is found among species (Torres and Vanni 2007; El-Sabaawi et al. 2012). Intraspecific variation in excretion often cannot be attributed to either organismal parameters, like diet and tissue stoichiometry, or environmental parameters, like productivity (Torres and Vanni 2007; McManamay et al. 2011; El-Sabaawi et al. 2012). Here, we consider one potentially important but largely unconsidered influence on the excretion rates of individual wild fish: variation in the strength of predation risk.

Our results show that predation risk substantially reduces N recycling of Trinidadian guppies by altering their size distribution, food consumption and protein catabolism. The mean estimated daily N excretion of guppies reared with predator cue was 39% lower than that of controls. Much of this reduction can be attributed to predator-exposed guppies being smaller (Figure S1a), as body size is a primary determinant of N excretion (Hendrixson et al. 2007). After correcting for the smaller size of cue-exposed guppies (see Torres and Vanni 2007), our average cue-exposed guppy still excreted 17% less N than a similarly-sized cue-naïve guppy (one-way ANOVA, $F_{1,63} = 19.3$, $p < 0.0001$). Some of this residual difference can be explained by variation in N consumption, as fish that consume smaller meals excrete less N (Lovell 1998) and cue-exposed guppies ate less than controls (Figure 1). After accounting for differences among treatments in fish size and food consumption, however, cue-exposed guppies still excreted 11% less N than controls (one-way ANOVA, $F_{1,63} = 4.5$, $p = 0.03$), likely due to digestive or other physiological trait change under predation risk. In total, these results indicate that predators change the N excretion of guppies by altering their life history (size), behavior

(consumption rate) and physiology, with the combined effect being a near halving of total N release by cue-exposed guppies. Studies seeking to understand variation in N excretion in wild fish may be improved by accounting for the predation risk facing study populations.

The changes in excretion observed in this study would likely translate into changes in ecosystem processes. In Trinidad, mesocosm studies have demonstrated that even small differences in excretion among guppy populations are associated with changes in ecosystem function (Palkovacs et al. 2009; Bassar et al. 2010; Bassar et al. 2012). Extending our results to the field, predation risk would reduce the flux of N from guppies to the stream. Because N limits productivity in many streams (Dodds and Whiles 2010), predators may reduce primary productivity by altering consumer excretion. It would be interesting to assess how the magnitude of this effect compares to and interacts with other predator effects on these stream ecosystems (*e.g.*, direct consumption, density-mediated trophic cascades, and non-consumptive effects).

Does reduced N-excretion reflect an adaptive response to predation risk?

In this experiment, altered N excretion by cue-exposed guppies reflected whole-organism change in how consumed N was used for physiological processes. Compared to controls, cue-exposed guppies excreted a smaller fraction of consumed N as ammonia (Figure 2c) and retained a greater fraction of consumed N in tissues (Figure 3b). Here, we interpret these results in the context of previously-demonstrated behavioral responses of wild guppies to predation risk to assess the potential adaptive significance of the physiological changes we observed in this study.

In nature, guppies feed less where predators are present and when predators are active (Fraser and Gilliam 1987; Fraser and Gilliam 1992; Dugatkin and Godin 1992; Fraser et al. 2004). These reductions in foraging activity incur substantial fitness costs, as predator-exposed fish have slower growth, lower reproductive output, and less time to allocate to reproduction

(Fraser and Gilliam 1992; Fraser et al. 2004). Lab studies consistently reproduce these patterns using chemical predation risk cues, as lab-reared guppies exposed to chemical cues feed less and grow more slowly than unexposed controls (Torres-Dowdall et al. 2012; Handelsman et al. 2013, this study).

Studies on the physiology of food restriction provide a set of expected physiological responses to such lowered food intake (reviewed in Wang et al. 2006) and can be used to predict how food restriction will alter nutrient recycling by food-stressed prey. Across a wide taxonomic spectrum, vertebrate animals faced with food restriction preferentially mobilize glycogen and lipid stores for energy production (Wang et al. 2006), increasing the C:N of their metabolism, reducing amino acid catabolism, and lowering the production of N waste as ammonia (Jobling 1980; Lyndon et al. 1992; Yang and Somero 1993; Mehner and Wieser 1994; Navarro and Gutierrez 1995; Alsop and Wood 1997; Forsberg 1997; Kajimura 2004; Wang et al. 2006; Luo and Xie 2009; Sinha et al. 2012; Liew et al. 2013). The adaptive significance of these changes is a sparing of the resource stores (*i.e.*, protein) most needed for future physiological activities (Cherel et al. 1992; Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010), thereby increasing the N retention efficiency of food-restricted consumers (Huggins and Munday 1966; Mayzaud and Conover 1988; Cherel et al. 1992; Company et al. 1999; Kousoulaki et al. 2010; Akpınar et al. 2011). We suggest that the reduced N excretion and increased N efficiency of predator-exposed guppies in this experiment represent a single case of a widespread response to food restriction that adaptively spares proteins from catabolism.

Predation risk meets food deprivation: balancing top-down and bottom-up forces

The results of our study diverge from a recently-proposed, powerful set of predictions for the impacts of predator-induced physiology on the nutrient cycling of prey (Hawlena and

Schmitz 2010a; Hawlena and Schmitz 2010b; Hawlena et al. 2012; Leroux et al. 2012). These predictions derive from the notion that conserved physiological responses of animal prey to predation risk drive qualitatively consistent changes in nutrient use and retention under predation risk. The authors describe that predation risk should increase metabolism and glucocorticoid expression, promoting protein catabolism for gluconeogenesis (Hawlena and Schmitz 2010a). As a result of increased metabolism, predator-exposed prey should prefer more C-rich foods, catabolize N stores to meet elevated metabolism, excrete N at a higher rate, and retain less N in tissues, increasing carcass C:N (Hawlena and Schmitz 2010a). These changes in nutrient recycling then translate into altered rates of ecosystem function (Hawlena et al. 2012). Results from the guppy system, however, are inconsistent with these predictions. In addition to the results from this study, cue-exposed guppies have lower glucocorticoid expression (Fischer et al. 2014) and unchanging (for high predation populations) or lower (for low predation populations) respiration (Handelsman et al. 2013).

We suggest that these differences between the guppy and grasshopper-spider systems reflect differing food availability and qualities in low-risk refuge habitats. Grasshoppers continue to forage on foods when sheltering from predators, enabling predator-exposed grasshopper prey to fuel the higher rates of metabolism and protein catabolism associated with predation risk (Hawlena and Schmitz 2010a). Moreover, grasshoppers sheltering from predators in refuge habitats can forage selectively on high C:N foods (Hawlena and Schmitz 2010a) that may be a better nutritional match to the higher metabolic C:N induced by predation risk. This change in diet quality alone can increase the rate and C:N of grasshopper metabolism (Zanotto et al. 1997), reinforcing differences in physiology and nutrient recycling between predator-exposed and naïve prey. In contrast, guppies have been widely shown to reduce feeding when predators are present

(Fraser and Gilliam 1987; Torres-Dowdall et al. 2012; Handelsman et al. 2013), titrating feeding opportunity against predation risk (Abrahams and Dill 1989). Unlike grasshoppers, moreover, wild guppies have limited ability to choose diet qualities. Guppies only effectively assimilate C and N from stream invertebrates (Zandona *et al*., in prep) that vary much less in their tissue C:N than do the primary producers consumed by grasshoppers (Sterner and Elser 2002; Zandonà et al. 2011; Leroux et al. 2012). In total, we expect that reduced feeding under predation risk and a lack of diet choice in shelter habitats prevents guppies from showing the elevated metabolism and rates of N excretion that would be expected based on predation risk-induced physiological change alone (Hawlena and Schmitz 2010b).

Guppies are just one of many consumers that trade-off predation risk against foraging opportunity (*e.g.*, Abrahams and Dill 1989; Brown and Kotler 2004). The physiology of any consumer that reduces food consumption under predation risk likely reflects a balance between the improved nutrient conservation of a thrifty, N-sparing metabolism and the enhanced predator encounter survival bestowed by a faster, protein-consuming physiology. Understanding how different organisms settle the conflict between predation risk and nutritional deprivation presents an opportunity to better understand the role of prey as nutrient recyclers. The quantity and quality of food in refuge habitat is likely an important mediator of the influence of predation risk on the physiology of prey, and, thereby, the role of prey as nutrient recyclers.

Conclusion

Like previous studies on grasshoppers and spiders, we show that predators substantially alter nutrient recycling by prey. In our study, however, nutritional limitation, not predation risk-induced physiological change, underlies the effects of predators on nutrient recycling by predator-exposed guppies. We propose that nutritional limitation and predation risk compete as

influences on aspects of prey physiology when prey reduce consumption under predation risk. The quantity and quality of food in refuge habitats likely is central to how prey resolve these competing influences on physiological trait expression.

ACKNOWLEDGEMENTS

We thank Jennifer Thaler and Michael Sheriff for organizing this special edition and for related helpful discussions to improve this manuscript. We thank Amy McCune, Nelson Hairston, Os Schmitz and one anonymous reviewer for comments that improved this manuscript and Corey Handelsman, Dale Broder and Cameron Ghalambor for discussions that helped shape this study. We are grateful for the help of Eugenia Zandona and Karen Sullam in collecting the fish used in this study, and we thank Connor Benfield, William Aragundi and Alicia Zhao for assistance with animal husbandry and Ezra Lencer, Amanda Cass and Amy McCune for use of laboratory resources. We thank the American Society of Naturalists, The Cornell Department of Ecology & Evolutionary Biology, the Andrew W. Mellon Foundation and a National Science Foundation DDIG for funding in support of this research. Christopher Dalton was supported by an NSF Pre-doctoral Fellowship for the duration of this experiment.

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TABLES

Table 1: Lipid, protein, carbon and nitrogen content of the high and low C:N diet (±S.E.). C:N are molar ratios.

Diet	% Lipid	% Protein	%C	%N	C:N
Low C:N (±SE)	10.0	48.0	48.87 (±0.02)	9.28 (±0.03)	6.14 (±0.01)
High C:N (±SE)	21.7	41.7	52.52 (±0.03)	8.10 (±0.04)	7.56 (±0.04)

FIGURES

Figure 1: (a) Total food consumed and (b) C and N consumed during the course of the experiment for high C:N diet (gray triangles), low C:N diet (black circles), no cue (filled symbols) and cue (open symbols) treatments (± standard error). Dashed lines in panel b indicate the C:N of the two diets (high C:N diet higher on y-axis than low C:N diet) and are the nutritional rails (Raubenheimer et al. 2009) onto which we forced guppies by restricting diet C and N composition. Sample size is sixteen for each treatment group.

Figure 1

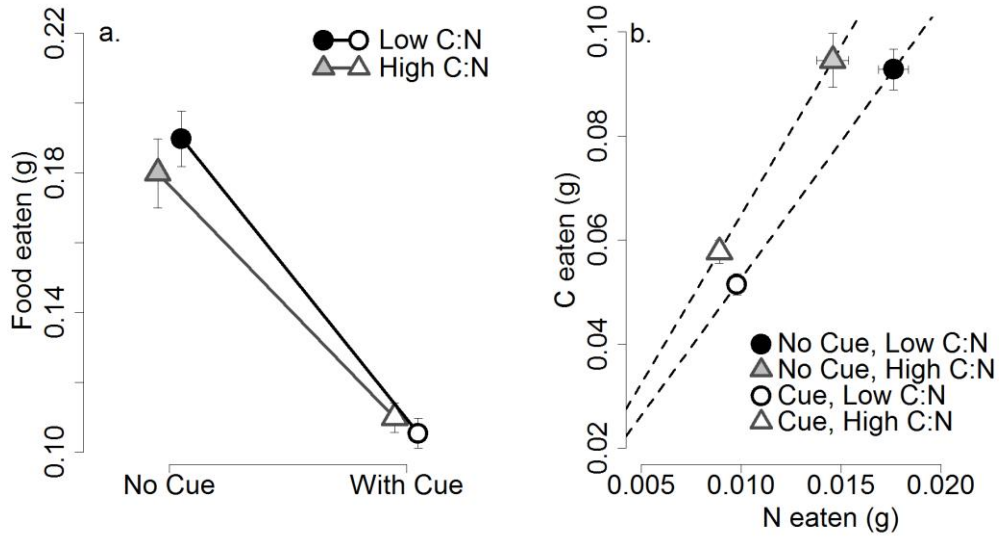


Figure 2: N excretion 1 hr post feeding (a), 8 hr post-feeding (b), and daily (c) for with cue (open symbols, dashed lines), no cue (filled symbols, solid lines), low C:N diet (black circles and lines) and high C:N diet (gray triangles and lines). Excretion rates 1 and 8 h post feeding have weight as a covariate. Total daily excretion has daily N consumption as a covariate. Lines represent best fit linear regressions of log-transformed excretion against the log-transformed covariate. Sample size is sixteen for each treatment group.

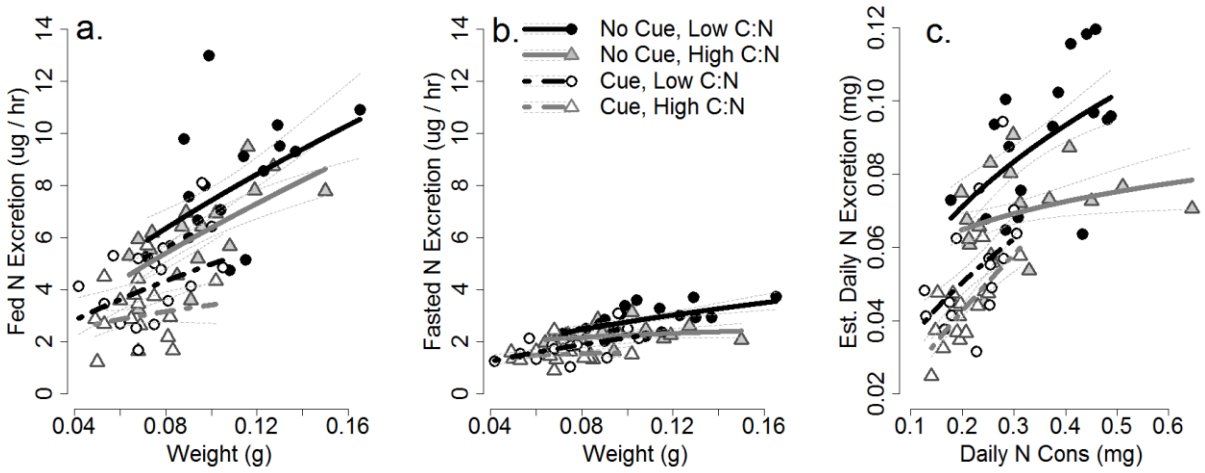


Figure 3: Estimated (a) carbon and (b) nitrogen use efficiency (± standard error) for guppies reared on high C:N diet (gray triangles) and low C:N diet (black circles) in the presence and absence of predator cues (open and filled symbols, respectively). (c) The ratio of carbon:nitrogen efficiency is the ratio of a:b and reflects the balance of the diet to the animal metabolic demands. Sample size is sixteen for each treatment group.

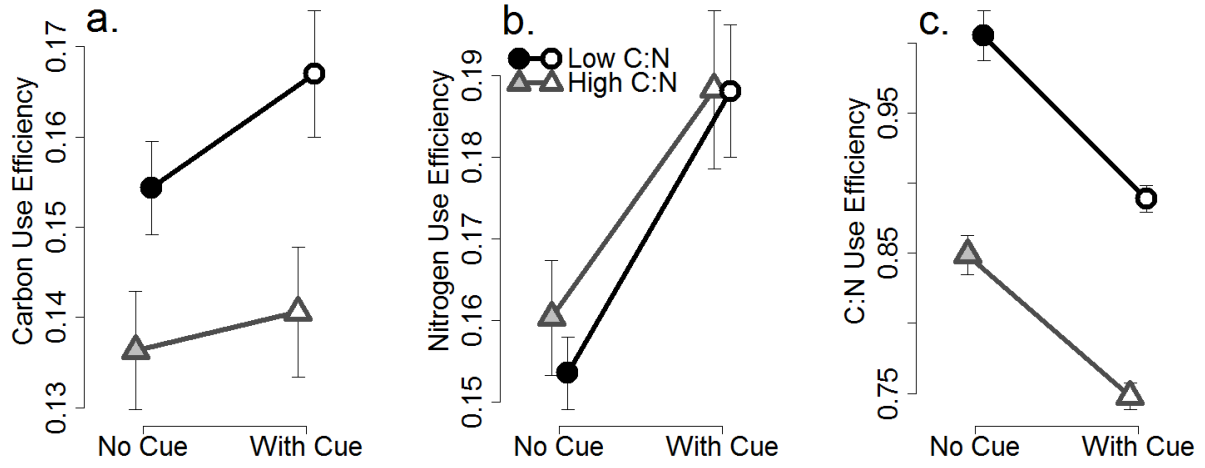
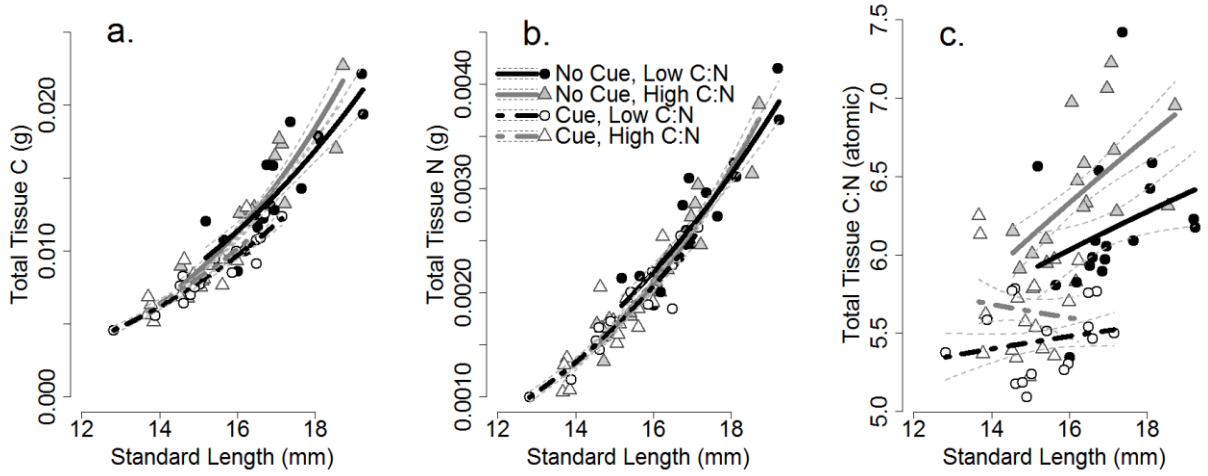


Figure 4: Estimated total tissue C (a), N (b), and C:N (c) of guppies as a function of standard length for with cue (dashed lines, open symbols), no cue (solid lines, filled symbols), low C:N diet (black circles, lines) and high C:N diet (gray triangles, lines) treatments. Lines are best fit regressions of log transformed nutrient content as a function of log-transformed standard length for each treatment group (±model standard error). Sample size is sixteen for each treatment group.



CHAPTER FOUR: GUPPY PHYSIOLOGICAL RESPONSE TO PREDATION RISK PARALLELS THE RESPONSE TO FOOD DEPRIVATION BUT IS ACCELERATED – FORESTALLING CONSEQUENCES OF EXTENDED FASTING

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ABSTRACT

Predation risk can alter the nutrient requirements of prey by inducing stress, thus altering the role of prey as nutrient recyclers. Food deprivation commonly results from predator-induced change in prey behavior, and can exacerbate or offset the physiological influence of predation risk. Disentangling food deprivation and predation risk effects on prey physiology thus remains a central challenge to understanding the ecophysiology of fear. Here, we assess the independent and interactive effects of food deprivation and predation risk on the nutrients incorporated in prey growth and tissues and released in prey wastes. We exposed Trinidadian guppies (*Poecilia reticulata*) to chemical cues from a predatory fish (*Crenicichla frenata*) that is known to induce trait change in guppies. During a 14 d trial, we measured food consumption, growth rate, tissue energy density, tissue stoichiometry, and nitrogen (N) excretion by guppies under predation risk and food deprivation. We found that variables related to the acquisition of N (tissue N content, N excretion, N efficiency) were strongly affected by the predation risk treatment, independent of food consumption. Variables related to biomass and energy retention (*i.e.*, growth rate, tissue energy density, carbon (C) acquisition efficiency, tissue C:N) were best explained by only the amount of food consumed. Predator cue did not influence these traits, outside of changes in food consumption by prey. These results suggest that predation risk triggers a physiological change in guppy N budgets that is different from the effect of food deprivation. We suggest this physiological change may be an adaptation to minimize protein loss from muscle in the face of predictable food restriction. Measurement of N excretion is thus a valuable trait for disentangling predation risk and food deprivation effects.

INTRODUCTION

The framework of ecological stoichiometry has garnered new insights at the intersection of physiological and ecosystem ecology by invoking the principle of mass balance (Sterner and Elser 2002). Because consumers can neither create nor destroy nutrients, researchers can predict the nutrients in a consumer's waste by quantifying consumer nutrient intake and sequestration into tissue. Ecological stoichiometry thus links mechanisms of resource acquisition (feeding morphology and behavior) and processing (digestive and metabolic physiology) to patterns of ecosystem function (availability of limiting nutrients). Researchers have used these connections to gain new insight into how food quality (*e.g.*, Moody et al. 2015) and metabolic processes (*e.g.*, Elser et al. 2003) can alter patterns of nutrient limitation in ecosystems (Sterner et al. 1992).

Analogous new insights have arisen at the interface of the animal physiological, predator-prey ecology, and consumer-mediated nutrient recycling (Sheriff and Thaler 2014). Dozens of research papers, many in the past five years, demonstrate that predators affect the metabolism of prey, changing their nutrient intake, sequestration, and waste production (reviewed in Hawlena and Schmitz 2010a; Zanette et al. 2014). This alters the availability of nutrients in the environment and can shape ecosystem function (Hawlena et al. 2012). The insight that predators can shape ecological dynamics by changing the role of prey as nutrient recyclers (Leroux et al. 2012), is adding a new dimension to our understanding of how the mere presence of predators can transform ecological dynamics (Preisser et al. 2007).

Leading the way down this new dimension, Hawlena and Schmitz have combined detailed field and lab experiments (Hawlena and Schmitz 2010b), ecological models (Leroux et al. 2012), and reviews of physiological studies (Hawlena and Schmitz 2010a) to describe what

may be a general phenomenon among prey facing acute predation risk. Imminent predation threat activates the prey's hypothalamic pituitary adrenal axis, stimulating production of glucocorticoid hormones that elevate metabolism, accelerate catabolism of amino acids for gluconeogenesis (Tomas et al. 1979; Hawlena and Schmitz 2010a), increase N excretion (McDonald and Wood 2004) , and decrease tissue N stores (Tomas et al. 1979). Aspects of this physiological response have been observed in prey spanning from grasshoppers to trout to humans, indicating that this response may be a highly conserved, general response of animals to their predators (Hawlena and Schmitz 2010a). The generality of this response makes this connection powerful, enabling prediction of nutrient cycling of prey based on a single environmental context (presence of predators)

When exposure to predation risk turns from acute to chronic, however, the adaptive physiological response to short-term predation risk incurs a substantial long-term nutritional cost. Elevated proteolysis and amino acid catabolism deplete tissue stores of proteins, and this depletion is accelerated by predator-stressed prey's preference for high energy, low protein foods (Hawlena and Schmitz 2010b). Moreover, while elevated glucocorticoid expression is associated with more food-seeking behavior (Tataranni et al. 1996; Kitaysky et al. 2003), many consumers decrease feeding activity under predation risk (Lima and Dill 1990), further exacerbating the fitness costs of consuming a low protein diet while catabolizing endogenous protein stores. The physiology of prey must thus balance the benefits of enhancing escape performance under acute predator exposure and the costs of depleting tissue reserves under chronic risk (Killen et al. 2011).

Laboratory studies of Trinidadian guppies (*Poecilia reticulata*) indicate that their physiological response to predation risk reflects food deprivation more than it does predator-induced physiological stress. Predator-exposed guppies feed less (Torres-Dowdall et al. 2012), decrease glucocorticoid expression (Fischer et al. 2014), do not change or decrease respiration (Handelsman et al. 2013), and decrease food consumption, N waste production, and tissue C (Dalton and Flecker 2014). These physiological responses are consistent with the physiological change induced by food restriction in vertebrate animals (Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010). Food consumption and predation risk, however, are confounded in prior studies of predation risk in Trinidadian guppies, obviating independent assessment of food deprivation and predation risk effects. Understanding the equivalence, or non-equivalence, of predation risk and food deprivation of drivers of different aspects of animal physiology is an important goal for understanding animal life histories (Capellán and Nicieza 2007; Zanette et al. 2014). Doing so, moreover, enables improved prediction of how environmental and evolutionary factors affect the nutrient excreted by animals in nature.

Here, we layered a food deprivation treatment onto the design used by previous studies to vary exposure of guppies to chemical cues released by their dominant diurnal predator, *Crenicichla frenata*. These chemical cues are known to induce behavioral, physiological, and life history trait change in guppies (Torres-Dowdall et al. 2012; Handelsman et al. 2013; Fischer et al. 2014). Our high and low food ration treatments enable independent assessment of predation risk and food deprivation as drivers of guppy physiology and nutrient cycling.

METHODS

Experimental Subjects

We collected guppies from two locations in both the Aripo and Arima Rivers of the Northern Range Mountains of Trinidad in March 2014. In the Aripo River drainage, guppies were taken from sites used for previous assays of guppies from environments with and without predators (Sullam et al. 2014). In the Arima River, guppies were taken from sites along a gradient from high to lower predation that was accessed from an entry point along Blanchisseuse Road (10.713421 N -61.293947 W). Pairs of male and female wild-caught guppies were kept in 3-L tanks in a modified recirculating system (Aquatic Habitats, Apopka, FL; 12:12 L:D photoperiod, temperature 25 ± 1°C), fed twice daily (hatched *Artemia* cysts), and checked daily for offspring.

Offspring were removed from breeding tanks on discovery and reared in 1.5-L tanks until individuals could be reliably sexed (>25 days post birth). The feeding ration was the same as the above. We reared each brood from a single male-female pairing in a single 1.5-L tank on an ad-lib feeding regimen until beginning the experiment. At the initiation of the experiment, virgin female guppies from each brood were anesthetized with MS-222. We verified the sex of each fish under a dissecting microscope and measured each for length and weight. Females were then randomly assigned to one of four treatments (see below).

Experimental Design

The experiment was a factorial design with two main effects: food ration (low vs. high ration) and predation risk (with vs. without predator cue). These four treatments were blocked spatially on a shelving unit, with each block containing only full siblings from the same brood, such that brood effects could not be distinguished from spatial blocking effects. We established 16 full blocks per treatment (see Supplemental Table 20 for information about population

composition of these blocks). The combined effect of brood and block is henceforth referred to as "block" effects, though this variation includes both the effect of the common rearing and genetic background of the brood and any consistent variation in the rearing environment due to the location within the shelving unit.

During the experiment, guppies were fed a diet of frozen *Mysis* shrimp (Hikari^{TM}), dried to constant mass at 55^{o}C and homogenized using a coffee grinder. Guar gum (Bob's Red Mill^{TM}) was added at 1.5% of dry weight as a binding agent to facilitate collection of uneaten food. The high food diet ration was designed to exceed the daily food consumption required by guppies for maximum growth (Reznick 1983; Dalton and Flecker 2014). The low food ration (75% lower than the high food ration) was set at a level below the reduction in food consumption induced by predation risk, ensuring the low food treatment provided a range of food consumption levels equal to or greater to the food deprivation of predation risk alone.

Immediately prior to each feeding, we warmed 1 g of each dry diet to room temperature and mixed the dry food with water at a 2:3 food:water ratio, such that the diet was 40% ± 0.4% (standard error of 20 replicate samples) dry weight. Each paste-like diet was then loaded into a luer tip micro-syringe (Hamilton, Reno, NV), and a known volume of the appropriate treatment food was delivered to each guppy. Guppies were fed two times per day as in studies of guppy life history and physiological responses to predation risk (Torres-Dowdall et al. 2012; Handelsman et al. 2013).

The predation cue treatment was introduced by the water plumbed through each guppy tank on a recirculating rack. Each 1.5-L guppy tank either received flow from a 120-L supply tank housing a single pike cichlid (*Crenicichla frenata)* or a 120-L supply tank with no fish in it.

Crenicichla is a dominant guppy predator, and previous studies have shown guppies respond strongly to cues released by this predator (Torres-Dowdall et al. 2012; Handelsman et al. 2013; Dalton and Flecker 2014). The *Crenicichla* in our experiment was reared on frozen brine shrimp. Guppies thus only received predator kairomones and no conspecific alarm cues. Both of the 120-L supply tanks received a constant in-flow of room temperature, dechlorinated tap water at a rate of 1 L min^{-1} to dilute predator cues and maintain water quality.

Response Variables

The duration of the experimental feeding and predator cue exposure was 14 days. On day 14, we fasted guppies for 12 h, conducted an excretion trial (see below), fasted guppies another 12 h to ensure emptying of gut contents, then euthanized guppies with an overdose of MS-222. After guppies were euthanized, we estimated length using handheld digital calipers, and estimated wet weight after blotting fish dry. Specific growth was then calculated as the difference in log-transformed initial and final mass divided by the number of days elapsed between measurements. After measurements were made, all tissues were dried to constant mass at 55°C and reweighed to estimate total dry mass.

We estimated food consumption by collecting uneaten food after each feeding using a modified plastic pipette. Food was collected 90 minutes after feeding to allow guppies time to finish consumption. Collected, uneaten food was placed on pre-weighed metal drying pans labeled for each fish and dried at 55°C between collections. The total dry weight of collected food was measured weekly, by allowing pans to dry to constant mass. Drying dishes were then ashed for 4 hours at 500°C and reweighed to estimate the ash-free dry mass (AFDM) of uneaten food collected. The loss of nutrient from the diet to leaching was corrected using a set of 8

fishless 1.5-L tanks that were plumbed, fed and food collected as if they contained fish (Supplemental Analysis 1). AFDM loss in these tanks was assumed to be due to leaching, and the recovered food from all other tanks was multiplied by the ratio of original food to recovered food from these tanks, thereby estimating the mass of food that would have remained had there been no leaching.

To estimate guppy tissue C and N content, the entire dry mass of each guppy was assayed for C and N content by weighing and tinning subsections of each fish for elemental analysis following methods similar to those in El-Sabaawi et al. (2012). Briefly, entire dried guppy carcasses were partitioned into chunks between 2-10 mg and weighed to the nearest thousandth of a mg. This method enabled us to directly assay the C and N content of, on average, 98% of the final dry weight of each fish. The percent C and N of each sample was assayed on a CNH analyzer (Vario EL III elemental analyzer, Elementar, Hanau Germany).

Guppies were stocked into the experiment on the afternoon of Day 0 and allowed to acclimate to the experimental conditions for two days. We assayed guppies for NH_4^+ excretion on Day 3. We removed guppies from their experimental tanks 60 minutes after delivering food , and we introduced them into opaque plastic beakers containing 500 mL of water from their source tank filtered to remove most bacteria (GF/F, Whatman, pore size = 0.7 µm). After a 30-minute acclimation period, we collected a subsample of the water in each beaker using a 60 mL plastic syringe. We collected a second sample from each beaker after another 60 minutes. On the same day, we also estimated excretion in a non-absorptive state (*i.e.*, after the influence of digestive processes) by collecting a second set of excretion samples after eight hours, again with a 60-minute incubation. The timing of each sample was dictated by prior pilot research, which

showed (i) 20 minutes of acclimation was sufficient to alleviate handling stress effects, (ii) the absorptive peak in NH_4^+ excretion was present for the first four hours after feeding, and (iii) post-absorptive metabolism was reached within six hours of fasting. No researchers were present in the sampling room between sampling time points, minimizing disturbance to the fish. This procedure was repeated on the final days of the experiment, though the fed and fasting observations were separated by a 12+ hour fast.

Subsamples for NH_4^+ analysis were filtered through an ashed filter (GF/F Whatman), refrigerated within 20 minutes of collection, and analyzed within 12 hours. NH_4^+ concentrations were measured on an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA), equipped with a UV filter (Holmes et al. 1999; Taylor et al. 2007).

Data analysis

We quantified food consumption by comparing uneaten food remaining in each tank to the total food fed to each fish. Starting with the total amount of AFDM recovered from each tank (see above), we multiplied recovered AFDM by the ratio of dry matter to AFDM in the original food to estimate the total amount of dry food uneaten by each fish over the experiment. By subtracting the total uneaten food from the total amount of dry food fed during the experiment (since each fish was fed a known quantity of food), we estimated total dry food consumption. We multiplied this value by the percent of C and N in the diet (analysis of six subsamples: 8.58±S.E.=0.09 % N; 39.62±S.E.=0.15 %C) to estimate total C and N consumption.

We calculated length-specific total C stocks, total N stocks, and tissue C:N of guppies by using log-transformed fish length as a covariate in regressions with log-transformed total tissue C, N and C:N. We used length as a covariate because dry weight, independent of length, can be

biased by variation in the tissue percent C (i.e., fish with more C in lipids have higher dry weight (Hartman and Brandt 1995)). To estimate C and N gain during the experiment, we created an estimate of initial C and N stocks for each guppy based on the initial percent of wet weight composed of dry weight (24.9±S.E.=0.01%), percent of dry weight composed of C (46.8±S.E.=0.4% C), and percent of dry weight composed of N (9.2±S.E.=0.1% N) of a set of 22 control guppies We multiplied the initial wet weight of each guppy by these percentages to estimate the initial C and N stocks in each guppy. We estimated the amount of C and N retained in tissues during the experiment by subtracting these estimates of initial C and N from the empirically measured total tissue C and N at the conclusion of the experiment.

We estimated the proportion of consumed N excreted by all guppies in the final week of the experiment. We assumed that the daily N consumption of a guppy on the day that excretion was measured matched the total consumption estimated over the previous seven days (estimated using the methods above) divided by seven days. We used our hourly absorptive and post-absorptive N excretion estimates to approximate total daily N excretion. We used previous data that indicated the absorptive peak in NH_4^+ excretion lasts for four hours after a meal and is followed by rapid, exponential decay in NH_4^+ excretion to post-absorptive levels by six hours post-feeding. We then estimated total daily NH_4^+ excretion as: (2 meals per day × 4 h per meal of absorptive metabolism × each fish's hourly absorptive NH_4^+ excretion) + (16 h per day of post-absorptive metabolism × each fish's hourly post-absorptive NH_4^+ excretion).

Statistics

All statistics were performed using R statistical software (R-Team 2010). Treatment effects on response variables were assessed using linear mixed models. The most complex,

biologically-feasible models were simplified to the best fit model using likelihood-ratio tests and corrected Akaike Information Criteria (AICc) score comparison. All models included "block" as a random effect to account for non-independence of individuals from the same brood and the non-independence of blocks which were arrayed spatially within the shelving unit.

The experiment was not powered to detect the effects of different environments of origin. We recorded the population of origin of each block and included this factor in analyses, but no treatment × population effects were significant in our statistical analyses. We thus consider all guppies in the experiment to reflect the general evolutionary history of all *P. reticulata*, not any particular intraspecific evolutionary lineage.

For all traits that scale allometrically, we used logarithmic transformation of both the response variable (*e.g.*, excretion rate, total tissue C stock) and covariate (e.g., fish wet weight, fish length). Suitability of data variance distributions to this analytic method was validated using the package gvlma on comparable models without random effects (Pena and Slate 2006) and by visual inspection of plots of variance distributions and residuals. We interpreted p values < 0.05 as significant and $0.05 – 0.10$ as marginally significant. We here present results based on AICc-score based model comparison and likelihood ratio tests. The details of the model comparisons are presented in Supplemental Tables.

We calculated effect sizes for traits directly measured in this experiment (growth, tissue energy density, tissue stoichiometry, excretion rates) using Hedge's *d* and associated confidence intervals (Nakagawa and Cuthill 2007) by using our high food, no predator cue treatment as the *control treatment* and dividing differences in treatment means by pooled standard deviations for

high food with predator cue (predator cue effect size), low food with no predator cue (food ration effect size), and low food with predator cue (combined effect size) treatments.

RESULTS

Growth and Energy Density

Variation in specific growth rate was best explained by a model with negative effects of both predation risk and food ration. (Figure 1A; Supplemental Table 1). The dry weight to wet weight ratio of guppies at the conclusion of the experiment, a proxy for tissue energy density (Hartman and Brandt 1995), was reduced by predation risk under high food conditions, and by food ration under no predation risk conditions (Figure 1B; Supplemental Table 2). The low food with predation risk treatment, however, was not different from the low food no predation risk treatment (treatment difference estimate = -0.001 ± S.E. = 0.003).. Log-transformed food consumption by guppies was reduced by both the predation risk and food ration treatments (Figure 1C; Supplemental Table 3).

We used measured food consumption as a covariate in analyses to assess whether treatments influenced growth and energy acquisition after accounting for treatment effects on food consumption (Supplemental Table 4-5). Specific growth and tissue energy density (dry weight : wet weight) were best explained by models with only a positive correlation with the measured food consumption. Incorporating treatment effects did not improve model explanatory power for tissue growth or energy density, after the treatment effects on food consumption were incorporated as covariates in these analyses.

Tissue Stoichiometry

We regressed total tissue C and N against standard length to assess variation in the allometry of C and N stocks with fish length, avoiding the confounding influence of percent of dry weight and nutrient ratio metrics (Dalton *et al*. 2014). The size of guppy tissue C stocks at a given length was reduced on both the predator cue and low food ration treatments (Figure 2A; Supplemental Table 6). The best model for variation in tissue C stocks also included a standard length × predation risk interaction, but removing this term did not reduce model explanatory power (df = 1, χ^2 = 2.65, p = 0.10). The size of guppy N stocks at a given length was reduced by the low food ration treatment (Figure 2B; Supplemental Table 7). No other model for tissue N stocks received substantial support.

Tissue C:N increased with fish length and was reduced by predation risk and food ration level (Figure 2C; Supplemental Table 8). On the low food ration, however, predation risk had minimal effect on fish C:N (treatment effect estimate = -0.03 ± S.E. = 0.02), and, thus, model explanatory power was improved by including a predator cue × food ration interaction effect (LRT; df = 1, χ^2 = 4.72, p = 0.03).

Variation in length-specific tissue C and C:N was best explained by models with only food consumption (Supplemental Tables 9, 11), suggesting treatment effects on tissue C and C:N reflected only differences in food consumption. In contrast, the best model for length-specific tissue N included a predator cue × food consumption interaction effect (Supplemental Table 10). Length-independent N stocks increased with food consumption (slope = 1.75 ± S.E. = 0.31), and length-independent N stocks increased more quickly with food consumption under predation risk (slope difference = 0.74 ± S.E = 0.35). This result suggests predator-cue exposed guppies more efficiently retained consumed N, a pattern we explore in more detail below.

C and N Retention Efficiency

We assessed the efficiency with which guppies converted the nutrients in their food to new growth by regressing the C and N gained during the experiment against the amount of C and N consumed. C gain scaled consistently with C consumption, and no treatment effect or interactions between treatments and C consumption significantly altered this relationship (Figure 3A; Supplemental Table 12).

N gain scaled positively with N consumption (slope = 0.075 ± S.E. = 0.01), and, sequestration of N into tissue was accelerated by predation risk (change in slope of N gain vs. N consumption = 0.020 ± S.E. = 0.007; Figure 3B; Supplemental Table 13). The best model also included a fixed treatment effect of low food rations, with guppies reared on low food rations having higher N gain at any level of consumption. No other model received considerable support, and the results indicate that guppies reared with predator cue or on low food rations more efficiently convert consumed N into tissue N.

Excretion measurements

Nitrogen excretion (log-transformed) scaled with fish wet weight (also log-transformed) at each measurement period (Supplemental Figure 1; Supplemental Tables 14-17). Below, we assess the treatment effects on N excretion by guppies in each measurement period, though all models included log-transformed wet weight as a covariate (as visualized in Supplemental Figure 2).

Predation risk reduced excretion in three of the four measurement periods (Figure 4; Supplemental Tables 14-17). Predation risk and weight alone best explained variation in excretion immediately after feeding at the beginning of the experiment. Other more complex

models also received substantial support, but all included a negative predation risk effect. At the outset of the experiment, predation risk did not explain variation in excretion after fasting (Supplemental Table 15; Figure 4B), but predation risk was associated with lower excretion both immediately after feeding and after a 12-hour fast at the end of the experiment (Supplemental Tables 16-17; Figure 4C-D; Supplemental Figure 1B-C).

Low diet rations only significantly reduced guppy N excretion in the fasted state at the end of the two-week period (Supplemental Tables 14-17; Figure 4D). Adding a food ration effect to the best model for either fed or fasted excretion on Day 1 did not significantly increase either model's explanatory power (LRT; Fed: df = 1, $\chi^2 = 0.15$, p = 0.696; Fasted: df = 1, $\chi^2 = 0.27$, p 0.60). At the end of the experiment, food ration effects were included in the best model for fed N excretion (Supplemental Table 16), however, removing both the food ration and food ration × fish weight interaction effects did not reduce model explanatory power (LRT: df = 2, $\chi^2 = 5.28$, p = 0.071). Low food rations did lower size-specific N excretion after a 12 h fast at the conclusion of the experiment (Figure 4D; Supplemental Table 17). The effect of low food rations thus only influenced guppy excretion after an extended period of low food rations and only in the non-absorptive state.

We regressed estimated N excretion per meal against estimated N consumption per meal during the second week of the experiment (see methods) to assess how much of consumed N was excreted as NH_4^+ waste (Figure 5; Supplemental Table 16). N excretion increased with N consumption, but with a power scaling coefficient much less than 1 (estimate = 0.575 ± S.E. = 0.089). This concave-down scaling indicates that guppies that consumed more N excreted proportionately less of that N.

After accounting for this power scaling, predator cue exposed guppies excreted less N than controls at any level of N consumption, indicating these guppies lost a smaller fraction of consumed N as ammonia waste (as suggested by growth efficiency measures above). Guppies on the low food ration had a higher intercept of the relationship between N consumption and N excretion.

Treatment Effect Sizes

We estimated treatment effect sizes for food consumption, growth, tissue energy, tissue stoichiometry, and excretion using Hedge's *d* (Nakagawa and Cuthill 2007). The effect sizes for excretion suggest a strong role for predator cues, independent of patterns in food consumption (Figure 6). During the first week of the experiment, only predation risk had a strong effect on excretion by guppies immediately after a meal. Effect sizes of the food treatment were not significantly different than zero during the first time period. At the end of the experiment, predation risk was still the primary cause of variation in excretion after feeding, though both predation risk and food deprivation contributed to variation in excretion after a twelve hour fast. In contrast, effect sizes for measures of growth and energy retention (guppy growth, tissue energy density, and tissue stoichiometry) were all strongly influenced by food treatment and less strongly affected by predator cues (Supplemental Table 19; Figure 6). Similarly, total food consumption was strongly affected by food ration level and more weakly affected by predator cues. Patterns of treatment effects on growth, tissue energy density, and tissue stoichiometry all seem to follow food consumption, as all show strong effects of food ration level and weaker effects of predation risk.

These results suggest that guppy excretion rates were largely decoupled from the nutritionally driven influences of food deprivation on growth and tissue energy storage.

DISCUSSION

In this study, we probed the mechanism underlying previously observed predation risk effects on nutrient cycling by Trinidadian guppies. Our results suggest that N processing by guppies more strongly reflects predation risk than food deprivation, while predator cue effects on energy retention and growth are straightforward byproducts of reduced food consumption under predation risk. Here, we explore this result and its implications for the role of prey in ecosystem-scale nutrient cycling.

Disentangling effects of predator cues and food deprivation

Previously, in a seven-week exposure of guppies to predation risk cues, we demonstrated that chronic exposure to predation risk reduced N excretion by Trinidadian guppies by 42% (Dalton and Flecker 2014). Due to the extended length of this exposure, however, our predator cue treatment was entirely confounded with the amount of food consumed by, and the body weight of, experimental guppies, obfuscating the mechanism underlying predation risk effects on guppy nutrient recycling. Was guppy physiology altered directly by the presence of predator cues, or did the physiological changes reflect sustained food deprivation or smaller body size induced by chronic predation risk?

As in our previous research, in the current study, predation risk reduced food consumption by guppies by 38% (on the high food ration) and 36% (on the low food ration). In the current study, we paired this predator-induced reduction in food consumption with a low food ration that reduced guppy food consumption by 69%-70%. We thus measured guppy

physiology with and without predation risk across a comparable range of food consumption, enabling independent assessment of measured food consumption and predation risk as influences on guppy physiology.

This approach revealed that guppy N budgets were differentially affected by predation risk and food deprivation. Predator cues immediately reduced post-consumptive N losses by guppies, whereas food deprivation did not influence N losses until the end of the experiment (Figure 4; Supplemental Figure 2). After 2 weeks of food deprivation, predator cue still more strongly reduced post-consumptive N loss than did low food rations (Figure 4c), even though the low food ration more strongly reducing guppy intake of N as food (Figure 6).

In contrast, variation in specific growth rate, tissue energy density, tissue C stores, tissue C:N, and C retention efficiency were all best-explained by models that included only an effect of measured food consumption, *per se* (Supplemental Tables 4-5; Figure 6). Notably, these measures all describe aspects of guppy biomass and energy content. For these energy-centric metrics, the predator cue effects reflect a straightforward influence of food restriction, no different than other causes of food deprivation.

These changes in N excretion affected the N retention efficiency of predator-exposed guppies. Guppies exposed to predator cues retained N more efficiently than predator naïve controls (Figure 3B), and these cue-exposed guppies maintained comparable levels of tissue N as cue-naïve guppies despite consuming nearly 40% less N during the experiment. At the conclusion of the experiment, predator cue reduced consumption-specific N excretion, while food restriction, *per* se, increased consumption-specific N excretion, despite reducing N excretion overall. Below, we consider the possibility that the predation-risk driven changes in N

excretion reflect an adaptive metabolic and digestive syndrome associated predator-induced food restriction.

Adaptive significance of predator-induced trait change

Most studies of predation risk effects on prey physiology have focused on prey that elevate glucocorticoid hormone levels and metabolic rates under predation risk (Hawlena and Schmitz 2010a). The often presumed and sometimes demonstrated adaptive significance of these metabolic changes is improved anti-predator behavior (Thaker et al. 2009; Hossie et al. 2010; Strobbe et al. 2010; Barreto et al. 2014), but these changes come at a nutritional cost. Prey that elevate glucocorticoid expression have higher blood glucose demands, accelerated amino acid catabolism to support gluconeogenesis (and thus elevated N waste), and depleted tissue protein content (and thus higher tissue C:N) (Hawlena and Schmitz 2010b). Higher glucocorticoid expression is correlated with stimulated appetite and feeding behavior (Dallman et al. 1993; Kitaysky et al. 2003), preference for higher energy content foods (Hawlena and Schmitz 2010b), and deposition of short term energy stores in liver glycogen (Dallman et al. 1993), possibly to support this altered nutritional demand. In total, these studies indicate that predation risk accelerates metabolism and especially N loss, rapidly depleting tissue N stores while maximizing energy gain and storage.

In contrast, the results from Trinidadian guppies, including this study, suggest predation risk induces an N-conservative physiological state. Predator cues reduce guppy glucocorticoid levels (Fischer et al. 2014), do not change or reduce metabolic rates (Handelsman et al. 2013), spare catabolism of amino acids, reduce N excretion, and decrease tissue energy density and C:N (this study; Dalton and Flecker 2014). These responses are consistent with the adaptive

physiological syndrome associated with chronic food restriction, which lowers metabolic rates, decreases N catabolism to conserve protein, and depletes tissue C stores (Navarro and Gutierrez 1995; Wang et al. 2006). In our study, the parallel direction of predation risk and food deprivation effects on all measured variables supports the conclusion that food deprivation is the causative agent of trait change in guppies under predation risk (Figure 6).

Despite the similarity of predation risk and food deprivation effects in this experiment, our excretion data suggest that predator-exposed guppies accelerate the metabolic syndrome associated with fasting, and, by doing so, spare more N from catabolism than would occur under food deprivation alone. Guppies under predation risk showed reduced N excretion immediately in this experiment (Figure 4A), whereas low food ration guppies did not show lower N excretion until the conclusion of the experiment (Figure 6). Predation risk effects on N excretion, moreover, were strongest in the post-feeding, absorptive state. This more rapid reduction in excretion under predation risk, especially immediately after feeding events, may explain how predator-cue exposed guppies could maintain comparable N stocks as predator-naïve guppies despite consuming 40% less food (Figure 2B).

The accelerated sparing of N by predator cue guppies may also explain why predation risk reduced consumption-specific excretion of N at the conclusion of the experiment, whereas low food rations, *per se*, elevated consumption-specific N excretion (Figure 5). Faced with food deprivation, most fish accelerate catabolism of C stocks in liver glycogen and perivisceral lipids to spare amino acids, thus decreasing N waste production and increasing waste C:N (Navarro and Gutierrez 1995). The traditional paradigm for metabolic fuel use during fasting suggests that sustained fasting only leads to catabolism of tissue N stores (and thus elevated N excretion)

when C stores have already been depleted by extended fasting (McCue 2010). Only at this later stage of fasting is N excretion expected to increase (Jobling 1980), as we observed on the low food treatment. Such depletion of tissue N stores could be especially costly under predation risk, as the first N stores to be catabolized under fasting stress are those in white muscle, (Loughna and Goldspink 1984) which are necessary for burst swimming performance.

We posit that guppies initiate the metabolic response to fasting immediately upon exposure to predator cues as an adaptive, anticipatory response to predator-induced food restriction. Reduced feeding under predation risk is a nearly universal response of guppies to predation risk (Fraser and Gilliam 1987; Fraser et al. 2004; Torres-Dowdall et al. 2012; Dalton and Flecker 2014) that makes predator cues a reliable forewarning of extended food restriction. Guppies may then use these cues to initiate an adaptive metabolic syndrome that minimizes the fitness costs of fasting by prioritizing catabolism of C-based glycogen and lipid stores to spare N-based protein stores associated with muscles. Whether this physiological change is anticipatory or not, the general parallelism of food deprivation and predator cue effects observed in this study indicates that, in responding to predator cues, guppies are physiologically adopting a metabolic syndrome associated more with food restriction than acute predation risk stress.

Results from Trinidadian guppies thus diverge from those of the metabolic stress paradigm (e.g. (Hawlena and Schmitz 2010a) but are consistent with the physiological response of consumers to food restriction. As reduced feeding under predation risk is a common indirect effect of predators on their prey (Abrahams and Dill 1989; Brown and Kotler 2004), it is likely that similar effects will be observed in other systems. Such a response, however, comes at its own cost. A high efficiency, fasting-based metabolism will lower blood glucose levels, reduce

stores of short term energy sources like glycogen, and incur reduced appetite and food consumption (Dallman et al. 1993), likely reducing growth rates.

Given that both predation risk and resource environment vary substantially across space and across time (Lima and Bednekoff 1999), we suspect that the deployment of different metabolic syndromes by prey that co-exist with predators will also vary with space and time to maximize the benefits and minimize the costs associated with each strategy in each environment. Few studies consider such spatiotemporal variation in predation risk, using instead binary experimental designs with "press" predation risk treatments (like that used in this experiment). Research into the eco-physiological effects of predators has produced results showing increases and decreases in the efficiency and nutrient waste released by prey (*e.g.*, McPeek et al. 2001; Stoks et al. 2005; Hawlena and Schmitz 2010c; Thaler et al. 2012; Miller et al. 2014), it seems the current challenge is to understand, in an environmentally realistic way, just where and when those responses are most likely to occur and whether they really will have the ecosystem effects that have been postulated.

Implications for consumer nutrient cycling

Intraspecific variation in rates of consumer-mediated nutrient recycling are immense and ecologically important (El-Sabaawi et al. 2012; Jeyasingh et al. 2014; El-Sabaawi et al. 2015), yet traditional models to explain variation in nutrient cycling have failed to predict this intraspecific variation. Neither food quality nor tissue nutrient content has proven to be a reliable indicator of the rate and ratio of consumer-driven nutrient recycling within species of fish (Torres and Vanni 2007; McManamay et al. 2011; Villéger et al. 2012).

We have previously suggested that predation risk is an important but rarely accounted influence on the excretion rates of fish (Dalton and Flecker 2014). Our own field survey supports this notion, as neither fish tissue nutrients nor fish diet explained variation in guppy field excretion rates, while the presence of the predatory fish, *Crenicichla frenata*, explained a significant portion of the variance in guppy excretion rates (Chapter 3). We suspect that further study will show such predator-driven effects on consumer nutrient recycling rates to be widespread.

Here, predation risk reduced excretion by guppies by 32% in total, by 26% per gram of tissue N, and by 15% per gram of consumed N. Thus, under predation risk, guppies were a smaller pool of N that turned over more slowly and released a smaller fraction of consumed N. Insofar as N can be limiting in streams (Dodds and Whiles 2010) and guppies can provide a major source of that N (Bassar et al. 2012), predation risk in this system should act to reduce rates of ecosystem function by decreasing the resupply of N by an important stream consumer.

Researchers, moreover, have identified the need to find traits that enable population managers and population biologists to assess the mechanism of food restriction of wildlife populations (Zanette et al. 2014). Such restriction may occur because of a general lack of resources in the environment, or because predators are denying prey the opportunity to feed. Based on the results of this experiment, we suggest that measuring N excretion and tissue N stocks in concert with other measures of energy or biomass may provide valuable information about the source of nutritional limitation on prey populations.

Conclusion

Predation risk reduces food consumption in guppies, as in many species of consumer. Our paired predation risk and food deprivation treatments enabled us to determine that many energy-related changes to guppy physiology reflected changes only in food consumption, whereas changes in tissue N processing reflected both food deprivation and predation risk. Guppies may accelerate a fasting metabolism under predation risk to spare tissue N, reducing their release of a potentially limiting substrate for primary producers. This physiological change is expected under reduced food consumption and may be widespread among prey that reduce consumption under predation risk. We encourage future researchers to assess the environmental conditions under which this starvation-induced metabolism is likely to occur in place of the widely demonstrated, acute predation risk metabolism.

ACKNOLWEDGEMENTS

We thank Amy R. McCune for comments that helped improve this manuscript. This research was funded by and NSF DDIG and an NSF FIBR Grant (DEB 0623632), a Cornell University Andrew W. Mellon Foundation Research Grant, and a Cornell Sigma Xi Society research small grant. We are indebted to Rana El-Sabaawi, Therese Frauendorf, Piatã Marques, and Misha Warbanski for assistance with field logistics and sampling and to M. Rich Moore for assistance with laboratory arrangements. Christopher Dalton was supported by an NSF Pre-doctoral Fellowship. We thank David Reznick and Joseph Travis for advice that helped inform the sampling and empirical design used in this experiment.

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FIGURES

Figure 1: Responses of growth rate (A), dry weight to wet weight ratio (B), and food consumption (C) to food ration and predation risk treatments. (B) Low food ration guppies (pink diamonds) grew significantly more slowly than high food guppies (blue squares), and predator-cue exposed guppies grew significantly more slowly than guppies without predator cues. (B) Guppies reared without predator cues on high food rations (left blue square) had significantly higher dry weight:wet weight ratios than any other treatment, reflective of higher tissue energy density. Guppies reared with predator cues on high food rations were lower in energy density, but were higher than either low food ration treatment. (C) Food ration and predation cue treatment effects both reduced food consumption. Large symbols reflect treatment means, while small symbols show replicate fish. Error bars reflect standard errors. Separation in the horizontal position is to enable comparison of individual points. N = 16 per treatment. HF = high food ration, LF = low food ration.

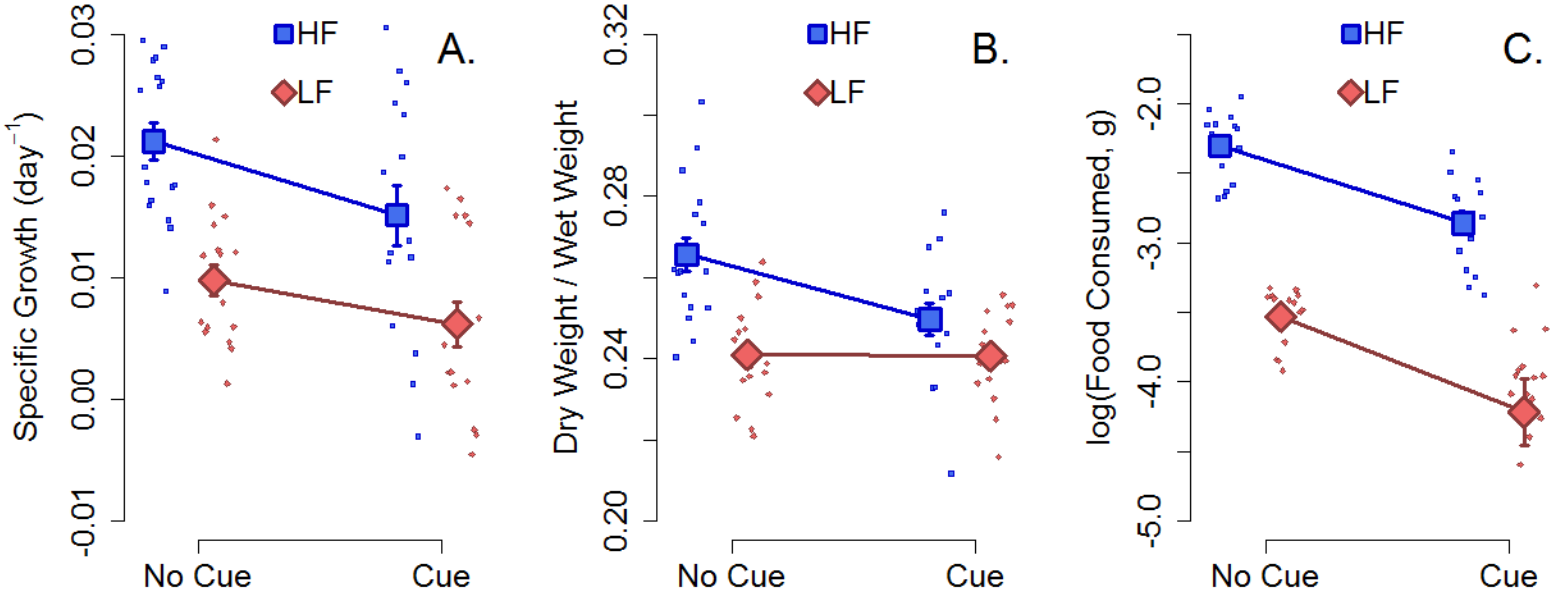


Figure 2: Total tissue C stocks (A), N stocks (B), and tissue molar C:N (C) at the conclusion of the experiment. (A) Both predator cue (light lines vs. dark lines and symbols) and food ration (blue vs. red lines and symbols) treatments reduced length-specific tissue C stocks, whereas (B) only food ration affected tissue N stocks. (C) Tissue C:N (molar) was higher for predator naïve high food ration guppies (dark blue squares and lines) than predator cue exposed guppies (light blue squares and line) on the high food treatment. On the low food ration (red and pink lines) tissue C:N did not differ between predator cue treatments. In all plots, thick lines represent best fit power regressions of each treatment, with the fish's final standard length as a covariate. N = 16 per treatment. HF = High Food ration, LF = Low Food ration.

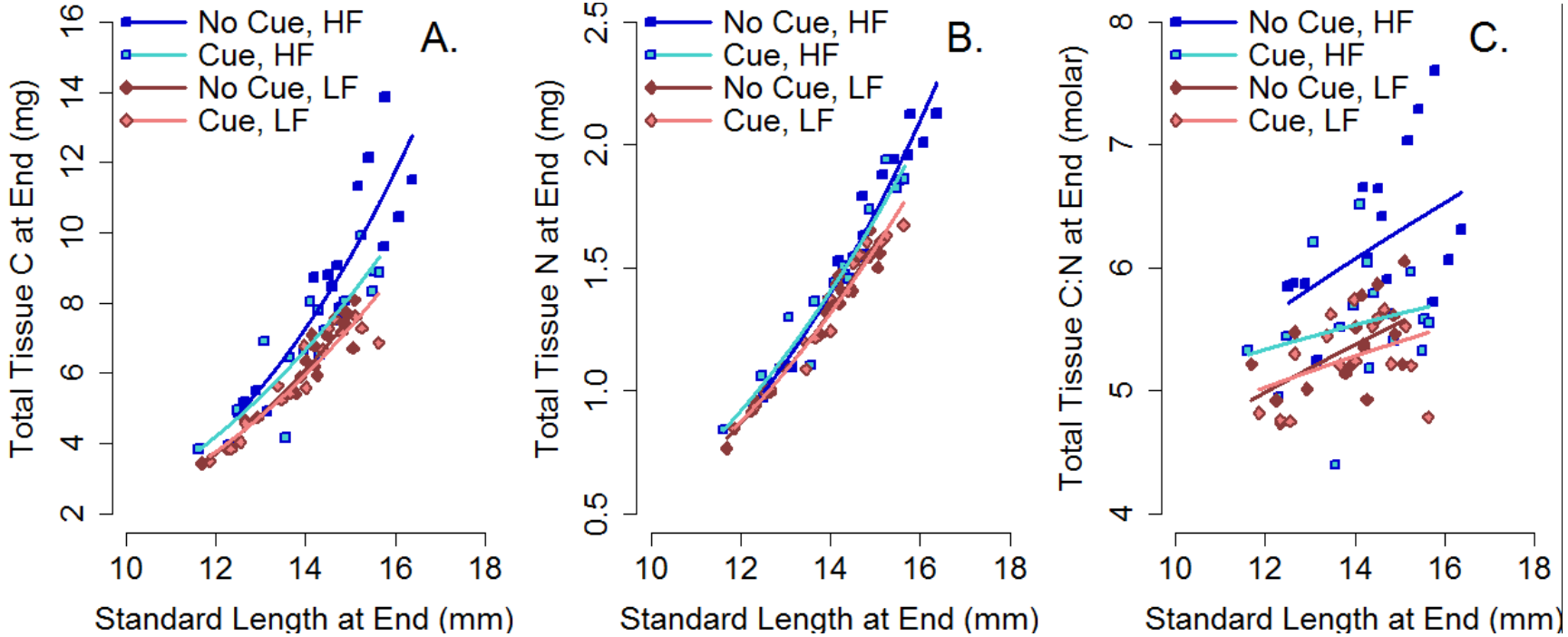


Figure 3: Gain of consumed C (A) and N (B) as a function of the consumption of C and N during the experiment. (A) C gain did not vary with any treatment and scaled positively with C consumption. (B) N gain scaled positively with N consumption, and this relationship was steeper in guppies with predator cues (light symbols and lines) than guppies without predator cue (dark symbols and lines), suggesting less of consumed N was wasted with predator cues. Guppies on the low food treatment (red or pink symbols and lines) also increased retention of N compared to high food treatment guppies (blue symbols and lines). In all plots, thick lines represent best fit regressions of each treatment, with the fish's consumption as a covariate. Thin dashed lines represent the standard error of the model fit. N = 16 per treatment. HF = High Food ration, LF = Low Food ration.

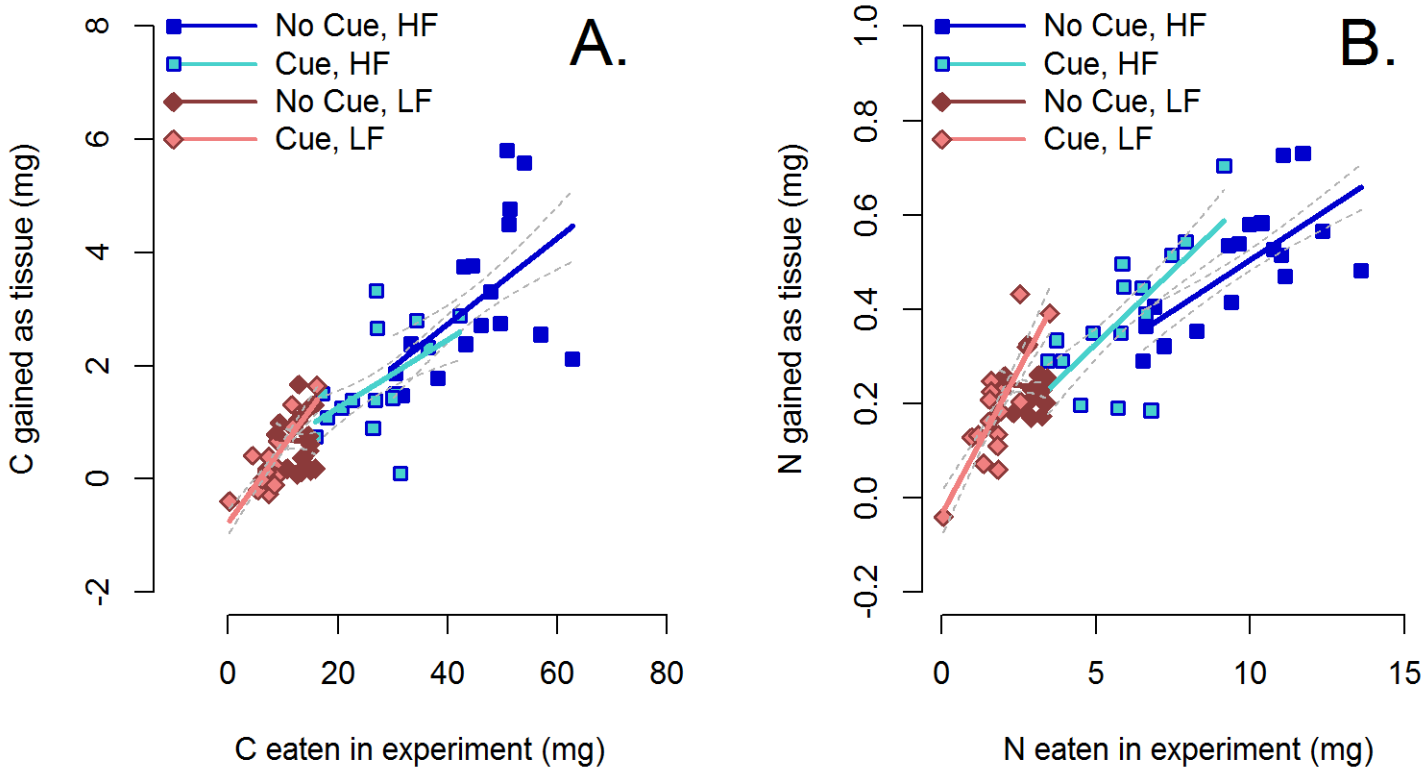


Figure 4: N excretion by guppies at the outset (A and B) and conclusion (C and D) of the experiment when guppies were recently fed (A and C) or fasted for more than 6 hours (B and D). (A) At the outset of the experiment, guppies on either food ration (blue vs. pink) excreted comparable amounts of N immediately after feeding, but excretion by both food levels was lower with predator cues than without. (B) After a several hour fast, treatment effects on N excretion were minimal. (C) At the conclusion of the experiment, both low food level (pink vs. blue) and predator cues reduced N excretion, but (D) the predator cue effect was much weaker after a 12 hour fast. Large symbols connected by lines reflect treatment means, while small symbols show the data for each replicate fish. Error bars reflect standard errors. Separation in the horizontal location of points is to facilitate comparison of individual points. N = 16 per treatment. HF = High Food ration treatment. LF = Low Food ration treatment. Units are µg N h^{-1} $g^{-0.75}$.

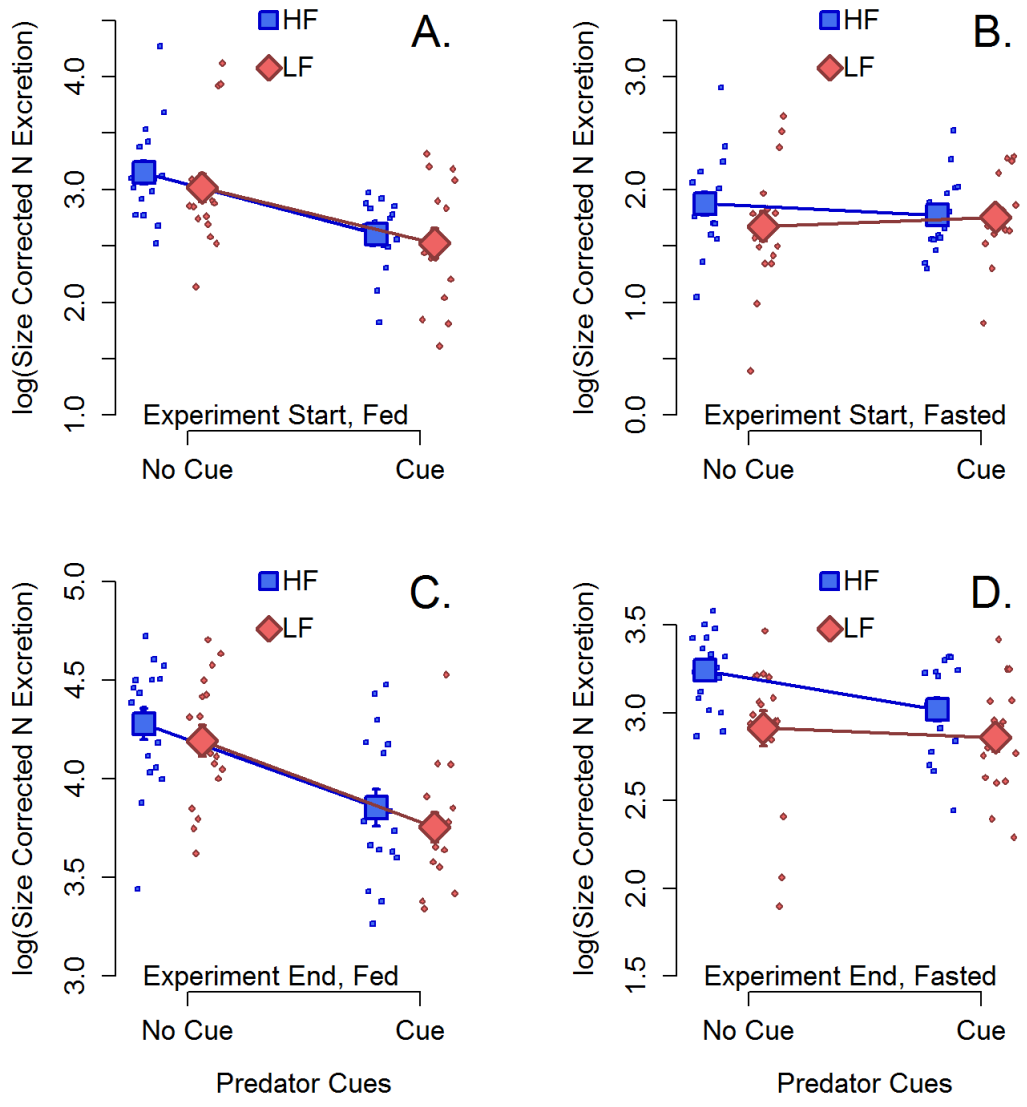


Figure 5: Estimated N lost as NH_4^+ excretion for each feeding event as a function of the amount of nitrogen consumed at each feeding event during the second week of the experiment. Guppies exposed to predator cues (light symbols and lines), had lower excretion than predator cue naïve guppies (dark symbols and lines) on both high food (blue symbols and lines) and low food (red symbols and lines) rations. N excretion was a decreasing function of N consumption, meaning fish that consumed more N excreted proportionately less of that N as ammonia waste. N = 16 per treatment. Thick lines represent best fit power regressions of each treatment, with the fish's final wet weight as a covariate. Thin dashed lines represent the standard error of the model fit. N = 16 per treatment. HF = High Food ration, LF = Low Food ration.

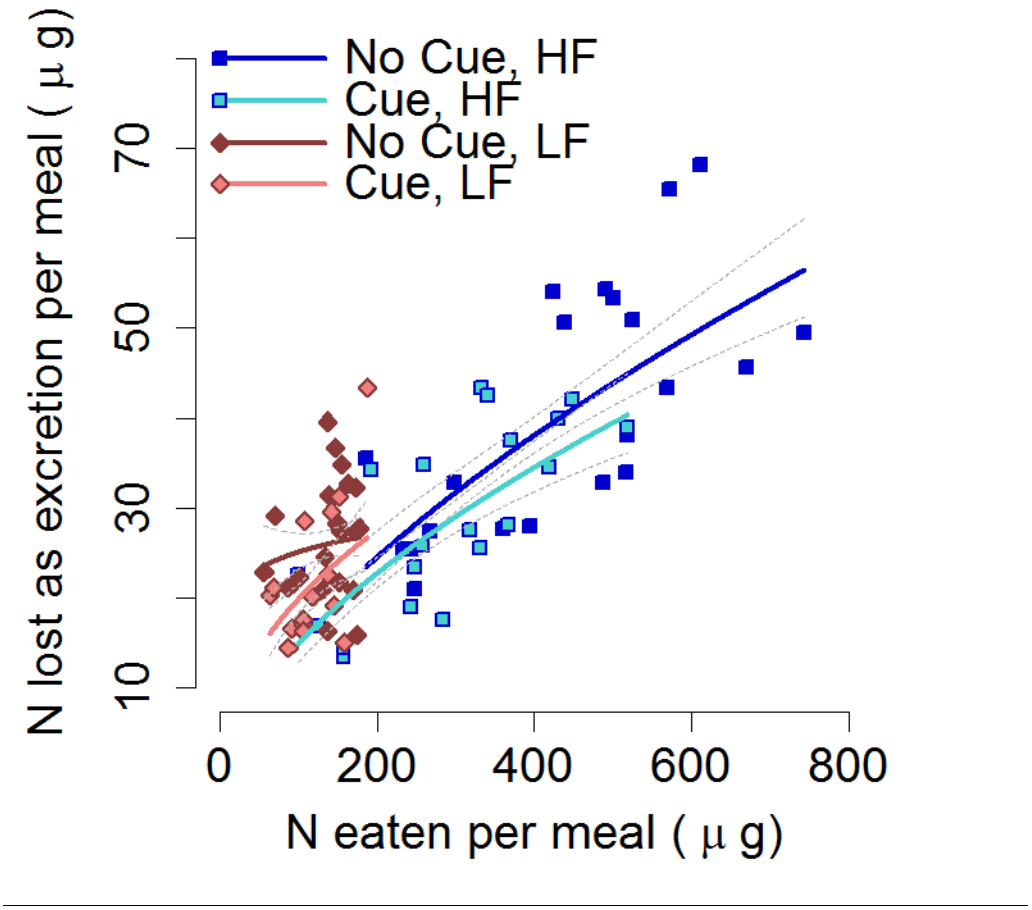
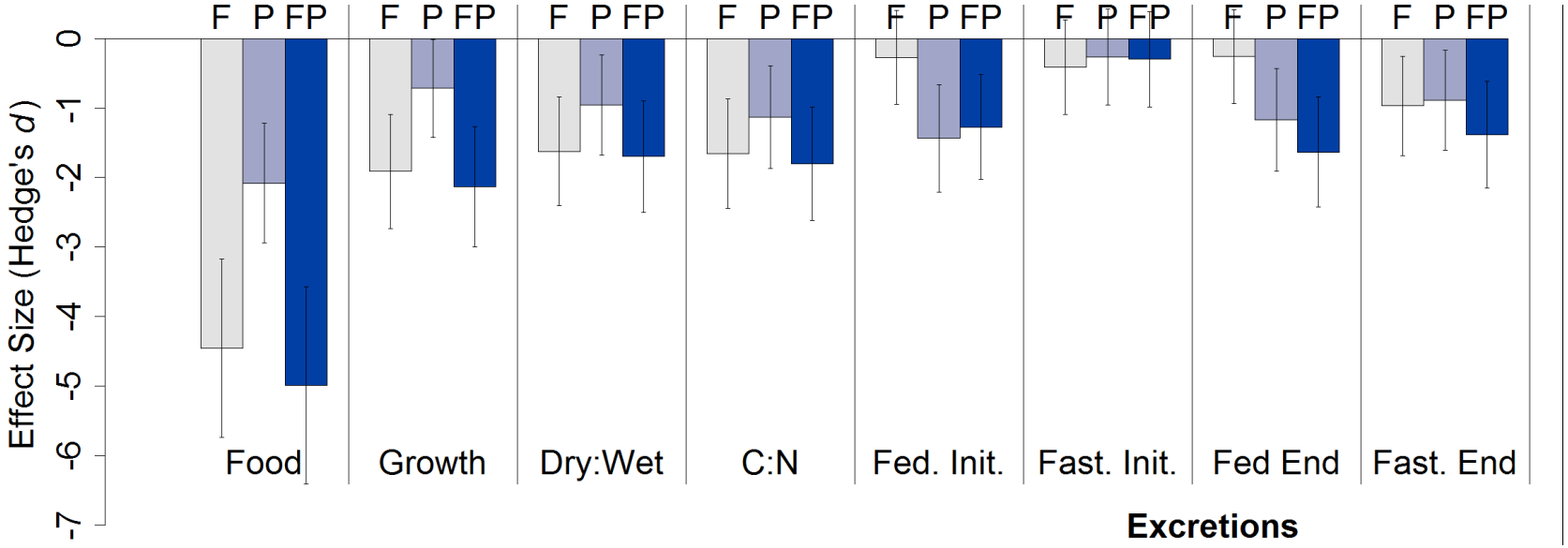


Figure 6: Effect size of low food ration treatment ("F", light gray bars), predator cue treatment ("P", light blue bars), and combined effect of both treatments ("FP", dark blue bars) on key response variables in the experiment. The relative magnitude of treatment effects sizes on food consumption ("Food"), specific growth, tissue energy density (Dry:Wet), and tissue C:N (molar) were comparable, with low food ration treatment effect sizes exceeding the effect sizes of predator cues. Predator cue and food ration effects were largely compensatory (*i.e.*, combined effect did not exceed effect of low food ration alone). In contrast, excretion measurements showed stronger predator cue treatment effects for all measurements except fasted excretions at the conclusion of the experiment. These results suggest predator cue effects on guppy *excretion* are largely independent of nutritionally-driven effects on other variables and can occur rapidly. For all measures, error bars represent 95% confidence intervals of measurements, based on methods in Nakagawa and Cuthill (2007). N = 16 per treatment.



CHAPER FIVE: PARALLEL AND DIVERGENT RESPONSES OF RESPIRATION AND PROTEIN METABOLISM TO PREDATOR CUES OVER VARYING TIMESCALES

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ABSTRACT

Predators can shape ecosystems by inducing stress in prey, thereby altering that prey's role in nutrient cycling. The influence of predator-induced stress, however can vary with the duration of prey's exposure, and it is unclear whether predator-induced plasticity is adaptive or a consequence of adaptive changes in other trait changes. Here we seek to disentangle the influence of short- and long-term predator exposures on plastic changes in nutrient recycling by Trinidadian guppies (*Poecilia reticulata*). We pair excretion measurements with measures of respiration to assess whether predator effects on excretion rate reflect altered rates of metabolism, in general, or change specific to protein metabolism. We reared guppies from birth to adulthood in water with or without chemical predator cues. We measured their excretion rate in water without predator cues to assess chronic exposure effects, independent of the immediate predation risk environment. We then measured the excretion and respiration of each guppy in water both with and without predator cues to assess short-term effects of predator cues. We found that any timespan of exposure to predation risk reduced guppy excretion by 25%, and this response did not differ between chronic and short-term exposure. The guppy excretion response to predation risk occurred quickly upon initiation of cue, and it relapsed quickly on cessation of cues. Reduced N excretion was correlated with increased N efficiency, as assessed by a stable isotope labeled diet. We suggest this highly reversible physiology enables guppies to calibrate their metabolism to the expected feeding opportunity in variable predation risk environments, reducing waste of proteins when feeding opportunity is limited.

INTRODUCTION

Ecologists have long sought to understand how predators affect the structure and function of ecosystems (Hairston et al. 1960; Holt 1977; Berryman 1992; Hairston and Hairston 1993). Predators influence ecosystems not only by altering the abundance of prey, through density-mediated trophic cascades (Paine 1980), but also by changing the traits of their prey, including prey behavior (Schmitz et al. 1997). Such trait-mediated indirect predator effects have been linked to changes in ecosystems, and these changes can be greater than those caused by predator-driven changes in prey density (Preisser et al. 2005). Describing trait-mediated indirect effects of predators is central to understanding ecological dynamics (Schmitz et al. 2004; Cortez 2011).

Researchers have only recently begun investigating a potentially important mechanism of indirect predator effects on ecosystems - changes in nutrient cycling by their prey (Hawlena and Schmitz 2010a; Leroux et al. 2012). The nutrients that prey release as excesses and byproducts of metabolism can alter the availability of limiting nutrients, thus changing ecosystem function (Vanni 2002; Capps et al. 2015). Predators induce stress in their prey (Hawlena and Schmitz 2010b), alter prey morphology (Tollrian 1995; Costello and Michel 2013), and change prey behavior (Schmitz et al. 1997). All of these changes can alter nutrient recycling by consumers (McIntyre and Flecker 2010), so predators may be central to determining the role of prey in ecosystem nutrient dynamics. Studies of such dynamics are just emerging but indicate predation risk can cause measurable change in ecosystem dynamics by changing prey nutrient demands (Hawlena et al. 2012).

Researchers have proposed two contrasting mechanisms for how predators may affect nutrient cycling by prey, and these two mechanisms yield largely opposite. One model, which

emphasizes the acute stress response to predation risk, predicts that predators should accelerate prey's N cycling by inducing prey to catabolize nitrogen (N) more rapidly to meet the immediate energy demands of the "fight or flight" response (Hawlena and Schmitz 2010b). The other mechanism, which emphasizes chronic food deprivation under predation risk, predicts prey will depress N cycling under predation risk to spare endogenous N stores from catabolism and thus preserve proteins in muscle tissue (Dalton and Flecker 2014). Both models draw on substantial evidence from laboratory models, and both rely on empirical measurements that are wholly consistent with theoretical predictions. These models are not mutually exclusive, and both likely occur in nature. Theory, moreover, enables only speculation about the conditions under which each mechanism is likely to be induced based on the postulated adaptive benefit of each.

The timescale of predator exposure may be a critical mediator of the prey's physiological response to predation risk. Rapid catabolism of N is likely adaptive for surviving immediate predation threats, because it provides cells with ready sources of energy (Hawlena and Schmitz 2010b). Sustained loss of tissue N, however, will deplete muscle N stocks, especially when accompanied by decreased food consumption under predation risk (Loughna and Goldspink 1984). Under restricted N intake, reduced catabolism of tissue N stores and thus lower N excretion may be adaptive (Navarro and Gutierrez 1995; Dalton and Flecker 2014). Much prior research demonstrates different physiological responses of prey to acute and chronic stressors (McEwen 1998; Steiner and Van Buskirk 2009), yet almost nothing is known about how the timescale of predation risk affects nutrient recycling by prey.

Here, we present the results of an experiment intended to disentangle the influence of long- and short- term predation risk on the physiology and nutrient cycling by prey. We use

Poecilia reticulata or, the Trinidadian guppy, a model species for the study of predator-prey interactions. We assessed the influence of chronic predation risk by rearing guppies from birth either in control (*i.e.*, predator free) or predator-cue containing water and then measuring the excretion rates of both groups in control water when the guppies had reached adulthood. We assessed the influence of acute predation risk by measuring the excretion rate of individual guppies in both control and predator-cue containing conditions after only a 24 h exposure in each.

METHODS

Overview

We here present three separate but related analyses. The first compares respiration and excretion rates of guppies from five populations varying in their evolutionary history with predators that were lab-reared with and without predator cues. In this analysis, all excretion and respiration measurements were made in water free of predator cues to isolate the effects of chronic predation risk during rearing. If chronic food deprivation is driving the response to predation risk, we expect predator-cue exposed guppies to show altered excretion rates even when tested in predator-cue-free water.

In the second analysis, we reared lab-bred descendants of three populations of guppies with varying evolutionary history with predators. As before, we reared guppies in water with and without predator cues to vary their exposure to chronic predation risk. We then measured respiration and excretion by each guppy in control water and predator-cue water. This analysis enables us to assess the influence of chronic predation risk, and short-term predation risk on respiration and excretion. If chronic food deprivation is driving the response to predation risk,

then the effect of short-term predator-cue exposure should be weak compared to that of chronic exposure (and not necessarily parallel).

The third analysis involved rearing guppies in water with and without predator cues for two weeks while feeding a diet enriched in ^{15}N, using the accumulation of the isotopic label to assess tissue turnover rates in guppies. If the plastic physiological response to predation risk is adaptive, we expect it will decrease catabolism of stocks of tissue N, resulting in slower accumulation of the isotopic label and less enriched tissue nitrogen. From this experiment, we can gain inference as to whether the plastic, nutrient-cycling response in to predation risk has the postulated adaptive benefit – reducing tissue N losses under restricted foraging.

Experiments 1 and 2 – Chronic vs. Acute Predation Risk Effects

Source populations

Source populations were those described in Handelsman et al (2013). We collected guppies from five (first analysis) or three (second analysis) populations in the Guanapo River basin of Trinidad. One population was collected in stream reaches where piscivorous fish impose high extrinsic mortality (Reznick et al. 1996a). We refer to this population as high predation (HP). A second population was collected in stream reaches where guppy predators are naturally excluded by barrier waterfalls. In such low predation (LP) environments across Trinidad's Northern Range mountains, the life history traits of independently derived LP populations have evolved convergently in response to relaxation of predation mortality (Reznick and Endler 1982; Reznick and Bryga 1987).

A set of three other populations were sampled from sites where experimental introductions of HP guppies had been made to stream reaches previously free of both guppies

and their predators. These introductions were established in 2008 and 2009, and guppies were collected from these experimental stream reaches two years after introduction. Evolution of life history traits in LP environments occurs rapidly, often within just a few generations (Reznick et al. 1997), so we consider these populations to be evolving towards an LP phenotype (Handelsman et al. 2013).

Rearing Conditions

To minimize maternal and other environmental effects, we reared all wild-caught guppies for two generations under common garden laboratory conditions (modified from (Reznick 1982)) in 1.5-l tanks (Aquatic Habitats, Apopka, FL) connected to a custom-made recirculating system and maintained on a 12:12 L:D light cycle at 27º C. Fish were reared on standardized food levels adjusted weekly for age and number of individuals per tank. In the morning, fish were fed Tetramin™ tropical fish flakes, (Spectrum Brands, Inc., Cincinnati, OH), and, in the afternoon, fish were fed brine shrimp nauplii (*Artemia* spp.). The quantity of food offered daily approximated *ad libitum* and was comparable to the high level of food administered by in previous studies (Reznick 1982). The breeding design is described in more detail by Handelsman et al. (2013).

Upon birth, second lab generation full-sibling broods were randomly assigned to two 1.5-L tanks (2–10 full siblings per tank) that differed in exposure to chemical cues from a predator (reared with or without cues from a predator) using a split-brood design. Siblings reared with cues from predators were reared in recirculating units that housed a pike cichlid (*Crenicichla frenata*) within the sump that supplied water to the tanks (Torres-Dowdall et al. 2012). Cues from predators included both kairomones and conspecific guppy alarm pheromones. Guppies

reared without cues from predators were housed in identical recirculating units without *Crenicichla* in the water supply. Second generation juveniles were anesthetized and sexed at 29 days (see above), and one male per family per rearing treatment was randomly selected and reared individually under the same conditions.

Experimental Design

For Experiment 1, guppies from one HP, one natural LP, and three introduced LP populations were reared in water either with or without predator cues. After these fish had reached maturity, we measured their respiration and excretion in water without predator cues. The difference between control guppies and those reared in predator-cue conditions thus only reflects the long-term influence of predation risk and not any short-term (*i.e.*, <24 h) response related to these cues (all incubations were conducted in predator-cue-free water after a 24 h acclimation).

For Experiment 2, guppies from one HP population, one introduced LP population, and one natural LP population were reared in water with and without predator cues and tested for excretion and respiration in water with and without predator cues. These results provide insight on how evolution, chronic exposure to predation risk, and short-term exposure to predation risk alter respiration and excretion rates of guppies.

Excretion and Respiration Rate Measurements

Methods for Experiment 1 were as described in Handelsman et al (2013). Briefly, 24 h prior to measurements, guppies were transferred into new tanks with predator-cue-free water. Guppies were not fed during this 24 h acclimation. After 24 h, each fish was transferred into a 100 mL static respirometer using water without predator cues, and acclimated for a minimum of

60 min (range: 60–70 min). Water current was permitted to flow through each respirometer during acclimation, but no water flow occurred during incubations. Respirometers were on shelves that were covered by an opaque blind throughout the experiment to minimize additional stress to the fish that could elevate respiration or excretion. A blank respirometer was measured along with each group of three fish to correct for microbial respiration, N uptake, and N release.

Oxygen concentrations of water samples were measured with a SI130 Microcathode Oxygen Electrode housed in a MC100 Microcell using a Strathkelvin 928 6-channel O_2 Interface connected to a PC running Strathkelvin 928 Oxygen System software (Strathkelvin Instruments Ltd, Glasgow, UK), as described by Handelsman et al (2013). Subsamples for NH_4^+ analysis were filtered through an ashed 0.7 µm filter (GF/F Whatman), refrigerated within 20 minutes of collection, and analyzed within 12 hours for HP and one Introduced population. Samples for all other populations were frozen within 30 min at -20ºC, slowly thawed in a refrigerator over 72 h, then assayed at room temperature for NH_4^+ . NH_4^+ concentrations were measured on an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA), equipped with a UV filter (Holmes et al. 1999; Taylor et al. 2007).

Both excretion and respiration rates were calculated by estimating the change in concentration of NH_4^+ or O_2, respectively, during the incubation, correcting for the volume of the respirometer and the duration of the incubation. This estimated O_2 consumption and NH_4^+ excretion per hour, which we then corrected for microbial processes by subtracting the estimated microbial respiration and excretion of a fishless respirometer. Microbial respiration and excretion were minimal, because we used a UV filter to sterilize the water continually prior to the incubation.

Excretion and respiration were corrected to account for differences in size among guppies. Both excretion and respiration rates scale with fish wet mass to a power less than 1, often shown to be approximately ¾ (Brown et al. 2004). To assess size-independent guppy respiration and excretion, we divided each fish's measured excretion and respiration rate by its weight raised to the ¾ power (Torres and Vanni 2007). We did not estimate scaling coefficients from our own dataset because treatment effects may influence both the covariate (fish weight) and response (respiration or excretion). This confounding of size and treatment would then bias our slope estimate, though results obtained using this method were qualitatively consistent with those presented here.

Excretion of N reflects catabolism of protein to supply energy for metabolism, so N excretion is expected to be correlated with fish respiration rate. We thus estimated respiration-independent excretion by guppies on all treatments by regressing all log-transformed excretion measures against all log-transformed respiration measures, then dividing measured excretion by measured respiration raised to the slope of this global relationship (Torres and Vanni 2007). Removing all interactions between treatments and respiration rate did not significantly reduce model explanatory power, suggesting this correction can be applied across all treatments (Experiment 1: likelihood ratio test (LRT): $df = 9$, $\chi^2 = 11.52$, $p = 0.24$; Experiment 2 LRT: $df = 11$, $\chi^2 = 18.3$, $p = 0.074$).

Experiment 3 – N turnover time estimates

Guppies in Experiment 3 were descended from guppies collected from pools along a 1 km reach of the Arima River. In the Arima River, guppies were taken from sites along a gradient from high to low predation that was accessed from an entry point along Blanchisseuse Road

(10.713421 N -61.293947 W). Adult male and female guppies were reared in 2.5L tanks following the methods described above.

We used first generation laboratory offspring for Experiment 3 because we were not comparing among populations and thus did not need to minimize maternal effects on expressed traits. Within 24 h of birth, first generation full sibling broods were transferred to a single 2.5 L tank in predator-cue-free water and fed following the regimen described above. After at least 10 weeks, all adult male guppies from individual broods were anesthetized, and two individuals from each brood were euthanized for analysis of initial tissue ^{15}N at the experiment outset for each family. Of the remaining male guppies, two individuals were randomly assigned to the predator-cue treatment and two individuals were randomly assigned to a control treatment. Of the two individuals from each brood assigned to each treatment, one individual was randomly assigned to be collected 7 d after the experiment onset, and the other to be collected after 14 d. Predator-cue guppies were reared in tanks connected to a 120 L source tank holding one *C. frentata*, while control guppies were reared in tanks connected to a 120 L source tank with no fish. For this experiment, the *Crenicichla* was fed only brine shrimp, so only predator kairomones, and not conspecific alarm cues, were used to induce predator effects.

For the duration of the experiment, guppies were fed an *ad libidum* quantity of a diet enriched with ^{15}N twice per day. This diet was created following the methods of Dennis *et al* (2010). The diet we fed to the guppies had $\delta^{15}N = 178.17 \pm$ S.E. = 14.1, whereas guppies at the experiment initiation had tissue $\delta^{15}N_i = 14.71 \pm$ S.E. = 0.07. Prior to harvesting, guppies were fasted to void all gut material, euthanized in ice water, measured for length and weight and then immediately transferred to a drying oven at 55ºC until constant mass. Guppies were then

subsequently ground to a homogenous powder, weighed into tins to 1 mg ± 0.05 mg, and measured for $\delta^{15}N$ at the Cornell Stable Isotope Laboratory (COIL).

We estimated tissue turnover rate by assuming exponential decay of initial N stocks towards equilibrium with the enriched stable isotope diet. The level of this equilibrium was derived from a previous empirical study that correlated diet discrimination factor at equilibrium with diet $\delta^{15}N$ in Trinidadian guppies (Dennis et al. 2010). The diet used in our experiment had an estimated diet discrimination factor of -105.12, meaning guppies at equilibrium with this diet would have an isotopic composition of 73.05 $\delta^{15}N$.

We modeled exponential decay following the approach used by McIntyre and Flecker (2006) to estimate tissue turnover in tropical fishes. In such models, the parameter *k* is used to estimate the fraction of endogenous N replaced each day. We estimated this parameter by modeling the decline of the difference between the equilibrium $\delta^{15}N$ of guppies on the enriched diet (Dennis et al. 2010) and the estimated initial $\delta^{15}N$ of each guppy, as described by Equation 1:

$$(1)\ (\delta^{15}N_f - \delta^{15}N_t) = (\delta^{15}N_f - \delta^{15}N_i)\, e^{-kt}$$

Where $\delta^{15}N_f$ is the equilibrium isotopic composition of guppies on the enriched diet, determined from Dennis et al (Dennis et al. 2010); $\delta^{15}N_t$ is the measured $\delta^{15}N$ of each guppy on day *t*, the day it was harvested; $\delta^{15}N_i$ is the estimated $\delta^{15}N$ of guppies at the experiment initiation, estimated for each family from the fish euthanized at the outset of the experiment; *k* is the scaling exponent (or decay rate), and *t* is the time since treatment initiation in days. Log-transforming this equation and rearranging enables us to solve for k for each fish using Equation 2:

(2) $k = [\ \log_e(\delta^{15}N_f - \delta^{15}N_t) - \log_e(\delta^{15}N_f - \delta^{15}N_i)\ ]\ t^{-1}$

We estimated this decay constant for all fish in the experiment at using $t = 7$ and $t = 14$ for fish collected 7 or 14 days after initiation of enriched diets, respectively. The adult male guppies used in this experiment did not change weight during the 7-14 d trial period, and there was no effect of predation risk on the weight change of guppies in the experiment (time effect = 0.002 ± S.E. = 0.003; predation risk effect = -0.002 ± S.E. = 0.004; intercept = -0.004 ± S.E. = 0.004). This result is not surprising, as male guppies show deterministic growth and would have ceased incrementing biomass before the experiment began. Because weight did not change for any treatment during the experiment, we assume that all enrichment during the experiment occurred due to turnover of existing stocks of N.

Statistical Analyses

We estimated treatment effects on standardized excretion and respiration rates and estimated values of *k* using corrected Akaike Score Criteria (AICc) based model selection. All models were implemented in R (R-Team 2010) using the package lme4. All models included "family" as a random effect to account for the split-brood design used in each experiment. Models for Experiment 2 also included fish identification as a random effect, since each fish was measured twice (once in predator-cue water, once in cue-free water). Suitability of data distributions to these analyses was assessed by implementing the package gvlma on linear models without specified random effects and by visual inspection of residuals. Effect sizes were estimated using Hedge's *d* (Nakagawa and Cuthill 2007).

RESULTS

Experiment 1: Population and chronic predator-cue effects on respiration and excretion

Guppies reared in predator-cue water had lower respiration rates (Figure 1; treatment effect estimate = -0.014 ± S.E. = 0.005; Table 1A; Figure 1A). In contrast, predator-cue rearing did not affect excretion rates. Instead, both size- and respiration- specific N excretion were affected only by the population of origin (Table 1B and 1C; Figure 1B and 1C). All populations had lower N excretion than the HP reference population, whether excretion was expressed as size-specific excretion (population differences ± S.E.: Intro Population 1 = -5.24 ± 0.79; Intro Population 2: -5.04 ± 0.71; Intro Population 3 = -1.15 ± 0.71; LP = -2.08 ± 0.84) or respiration-specific excretion (population differences ± S.E.: Intro Population 1 = -0.27 ± 0.03; Intro Population 2: -0.26 ± 0.03; Intro Population 3 = -0.07 ± 0.03; LP = -0.27 ± 0.03).

Experiment 2: Population, chronic risk, and acute risk effects on respiration and excretion

Weight-corrected respiration rate responded differently to the predator-cue treatments in each of the three populations (Figure 2A), meaning the best supported models for respiration included complex interaction terms (Table 1). Perhaps due to the low sample sizes, none of these models is very strongly supported (Handelsman et al. 2013), and removing these interaction terms only marginally reduces model explanatory power (LRT: df = 2, χ^2 = 5.30, p = 0.071). Focusing on models without interactions suggests effects of predator-cue on respiration are poorly supported by this dataset (Table 1). Respiration rate was only marginally less well-explained by models without a short-term predator-cue effect (LRT: df = 1, χ^2= 3.00, p = 0.08) or a long-term predation risk exposure effect (LRT: df = 1, χ^2 = 0.49, p = 0.48). In total, these results suggest respiratory responses to predation risk, especially short-term exposure, are inconsistent and weak.

In contrast, weight-corrected excretion rate was well explained by short-term predator-cue exposure and guppy population of origin (Figure 2B; Table 2B). Short-term predation risk exposure reduced guppy excretion rates (estimate =-3.43 ± S.E. = 0.59), while chronic predation risk exposure did not consistently alter guppy excretion rates. HP guppies had higher excretion rates than either the introduced or natural LP populations (introduced LP: treatment estimate = -2.31 ± S.E. = 0.79; natural LP: treatment estimate = -1.67 ± S.E. = 0.94). More complex models often received some support, but removing the additional terms did not significantly reduce model explanatory power.

Respiration-specific excretion was also lower in the presence of predator cues (estimate = -0.074 ± S.E. = 0.027; Figure 2C; Table 2C). HP guppies had higher respiration-specific excretion than either the introduced LP population (estimate = -0.093 ± S.E. = 0.031) or the natural LP population (estimate = -0.239 ± S.E. = 0.036). The effect of predator cues during incubation also reduced the excretion of Introduced LP guppies more than the HP guppies (cue presence × introduced LP population estimate = -0.084 ± S.E. = 0.038). Models including an effect of chronic predation risk did not receive substantial support.

Because the same fish were tested once in each water type, we can also test for the size of the change in excretion and respiration rates under short-term predator risk exposure. The best model for this difference in N excretion (*i.e.*, the effect of short-term predation risk) was not influenced by treatment or population but was significantly less than zero (-0.544 ± S.E. = 0.09, $p < 0.001$), meaning that the average guppy reduced its excretion by 0.544 $\mu g\ N\ h^{-1}$ when exposed to predator cues for less than 24 h. This reduction represents a 28% reduction in N excretion (compared with controls) caused by the short-term presence of predation risk. In

comparison, *per capita* respiration rate decreased by 2.4 μg O_2 h^{-1} (±S.E. = 10; p = 0.02), which is only 9% of the average respiration rate of control guppies (27.1 μg N h^{-1}).

The magnitude of treatment effect sizes (lumping all populations in Experiment 2) indicate weak but generally negative influences of long- or short-term predation risk on respiration, but strong effects of only short-term predation risk on excretion and excretion per respiration (Figure 3) The combined effect of long and short-term predation risk on guppy excretion was similar or smaller than the effect of short-term predation risk alone, and chronic exposure to predation risk minimally impacted guppy excretion.

Experiment 3: Estimating tissue turnover times in guppies

Estimates of *k* were only affected by the predation risk treatment (Table 3) and did not vary depending on whether the guppy was harvested 7 or 14 days after initiation of the labeled diet. This time invariance of the turnover rate (*k*) suggests this model of tissue turnover was not inappropriate for estimating the change in guppy isotopic composition with time. Tissue turnover was significantly slower under predator cues (0.032 day^{-1} ± S.E. = 0.006 under predation risk vs. 0.052 day^{-1} ± S.E. = 0.008 for controls), as reflected by the lower $\delta^{15}N$ of predator-cue reared guppies (Figure 5). This indicates predator-cue-exposed guppies assimilated less of the N from the high $\delta^{15}N$ diet (and thus having lower $\delta^{15}N$) (Figure 5). These estimated turnover rates give tissue N a median half-life of 22 days (range = 18 – 27 d) in predator-cue reared guppies but only 13 days (range = 12 – 15 d) in control guppies.

DISCUSSION

The results of this study provide a new vantage on the plastic responses of guppies to the chemical cues of their predators. As in previous studies, our results show that guppies reduce

excretion and respiratory rates in the presence of predator cues (Handelsman et al. 2013; Dalton and Flecker 2014). Unlike these previous studies, however, our results disentangle the influences of long- and short- duration exposure to predator cues. While long-term exposure to predator cues either did not affect or even increased guppy N excretion, short-term (*i.e.,* <24 h) exposure to predator cues reduced N excretion by an average of 25% of pre-exposure rates while only reducing respiration by less than 10%. This short-term effect was independent of the long-term exposure to predator cues. The potential fitness benefit of this change in N excretion may be reflected in 69% longer tissue retention of of N (and thus reduced demand for ingestion of new N) in guppies exposed to predator cues. Here, we consider these results in light of previous studies on predator-cue effects on guppies and prey in other systems, and we consider the potential adaptive significance of these patterns.

Contrasting influences of long-term and short-term predator-cue exposure

Our previous studies have found that Trinidadian guppies reduce excretion after exposure to predation risk over 3,14, and 49 days (Dalton and Flecker 2014, Dalton *et al*., in prep). Because these studies measured excretion rates by guppies in the same predation risk environment as the experimental rearing conditions, we could not independently assess the influence of chronic predation risk (*i.e.*, predation risk environment in rearing conditions) and the influence of acute predation risk (*i.e.*, predation risk environment in excretion test conditions). Acute and chronic physiological responses to predation risk can differ, directionally (McEwen 1998; McEwen 2004; Steiner and Van Buskirk 2009), so disentangling their influences on guppy excretion could highlight important short- and long-term consequences of exposure to predation risk. These different timescales of exposure, moreover, may explain the divergent predictions of

stress-based (Hawlena and Schmitz 2010b) and food deprivation-based (Dalton and Flecker 2014) models for predator effects on nutrient cycling by prey.

Our results highlight a limited role for chronic predation risk, by itself, as a driver of variation in excretion by guppies. Rearing guppies with predator cues for multiple months did not reduce guppy excretion relative to controls when the measurements were made after only a 24h acclimation in predator-free water. Thus, either chronic predation risk had no effect on guppy excretion rates, or those chronic effects were reversible within just a day of predator free-conditions. In contrast, as has been previously published (Handelsman et al. 2013), long-term exposure to predation risk was associated with decreased respiration rates even after the 24h acclimation in cue-free water.

Short-term predation risk, however, was the major source of variation in guppy excretion rates. Guppies reared in control conditions significantly reduced N excretion within 24 h of exposure to predator cues. In turn, guppies reared with predators cues significantly inceased N excretion within 24 h of removal for predator cues. The magnitude of the *decrease* in excretion by control-reared guppies when tested in predator-cue water was the same as the magnitude of the *increase* in excretion by predator-cue-raised guppies when tested in control water (control guppy decrease: estimate = 0.554 μg N $fish^{-1}$ hr^{-1} ± S.E. = 0.128; predator-reared guppy increase: estimate = 0.546 μg N $fish^{-1}$ hr^{-1} ± S.E. = 0.100). The strength of this short-term plastic response was not affected by the fish's long-term exposure treatment. Our results thus implicate a plastic response in guppy excretion rates driven by the presence of predator cues within the past 24 h that occurs independent of any longer term exposure.

This plasticity, moreover, occurs largely independent of patterns of energy consumption (measured as respiration rates). Because excretion of NH_4^+ reflects the byproduct of using amino acid catabolism for energy-yielding reactions (Kajimura 2004), reductions in NH_4^+ can occur either because total energy use declined or because the proportion of energy provided by amino acids declined (Mommsen and Walsh 1992). Here, our measurements of respiration-specific excretion suggest that the decline in N excretion under acute predation risk was driven by decreased reliance on amino acids as a metabolic fuel. Moreover, because these fish had been fasted more than 24h during the trial, excretion rates reflected post-absorptive release of endogenous stores of N (Gerking 1955; Dalton and Flecker 2014). In contrast, long-term, chronic predation risk did not affect respiration-specific excretion.

Measurement of N turnover time in these guppies gives some indication of the potential adaptive significance of reducing the fraction of metabolism provided by N-yielding amino acids. The N turnover rate of predator-exposed guppies was 40% lower than that of control guppies, increasing the half-life of N in their tissues by almost 70%. Such an increase in N retention would be adaptive in the face of predator-restricted foraging. Predators reduce feeding opportunities for guppies (Fraser and Gilliam 1987; Fraser et al. 2004; Torres-Dowdall et al. 2012), reducing the amount of food that guppies consume in experimental trials by 40% (Dalton and Flecker 2014). The general vertebrate animal response to food restriction is a decrease in catabolism of amino acids to maintain endogenous N stores in muscle (Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010). The results of this study further support the notion that the plastic response of guppy excretion to predation risk, reduction in size- and respiration-specific N excretion, reflects an adaptive physiological syndrome that maintains tissue N stores in the face of a predictable reduction in food consumption.

The rapidity with which guppies appear to transition from a predator-induced N metabolism to a predator-free N metabolism may reflect the need to calibrate physiology to the shifting predation risk environment of Trinidadian streams (Gilliam et al. 1993) in order to maximize the benefits and minimize the costs of each physiological state. Elevated N excretion is associated with enhanced amino acid catabolism that supports gluconeogenesis, with glucocorticoid hormones being a major control on the rate of this process (Hawlena and Schmitz 2010b). Such a metabolism is thought to be adaptive under predation risk because it increases escape performance (Thaker et al. 2009) and leads to increased food seeking behavior (Kitaysky et al. 2003) that can drive faster growth if resources are plentiful. When predation risk incurs food deprivation, however, such a metabolism would more rapidly draw down tissue stores of N in proteins that are critical for predator escape (Loughna and Goldspink 1984). Under predator-induced food deprivation, then, an N-conservative metabolism associated with suppressed glucocorticoid expression (Fischer et al. 2014) is likely adaptive (Navarro and Gutierrez 1995; McCue 2010), but comes at the cost of decreased appetite and thus growth (Tataranni et al. 1996).

The changes in N excretion that we observe in this study are consistent with broader metabolic syndromes associated with food deprivation and risk. Lower N excretion under predation risk is consistent with the lower glucocorticoid expression of guppies under predation risk (Fischer et al. 2014) reducing gluconeogenesis and thus preserving tissue N stores, as seen in our stable isotope labeling experiment. Lower glucocorticoid expression (and thus N excretion) would also be associated with decreased food seeking behavior (Kitaysky et al. 2003), which would itself be adaptive when food seeking incurs higher risk of predation events. In turn, when predators are absent and food abundant, (as it often is in sites with predators of guppies (El-

Sabaawi et al. 2012)), higher N excretion may occur because it is associated with the higher glucocorticoid expression of increased food seeking and increased anabolic tissue building (Mommsen 2001).

Reduced N excretion under predation risk, then may indeed be the result of selection for plastic increases in N retention under predictable, predator-induced food deprivation. Alternatively, this reduction may be the result of selection for plastic reductions in food seeking behavior under enhanced extrinsic mortality risk. That the two responses (food seeking and N metabolism) have a common hormonal basis (*i.e.*, glucocorticoid levels) makes disentangling these hypotheses difficult. Indeed, both may be acting simultaneously, but further investigation into the hormonal basis of N excretion under varying predation risk and food deprivation could yield promising insights.

Changes among populations and potential adaptive significance

We detected substantial differences among populations in respiration, excretion, and respiration-specific excretion rates. Because the fish used in this experiment were second generation, common-garden reared, differences among populations are presumably due to genetic differences (Reznick et al. 1996b). Our results provide a foundation for speculation about the potential adaptive significance of such genetic differences among populations. Importantly, in natural streams in Trinidad, gradients in predation risk environment are mapped upon parallel gradients in resource quality and quantity (Grether et al. 2001; El-Sabaawi et al. 2015a), meaning that any putatively adaptive trait differences between HP and LP guppies could also be due to selection from predation risk or from food quality.

Notably, in this study, both introduced and natural LP guppies had higher respiration rates and lower excretion rates than HP guppies (Figure 1; Figure 2). This results in LP guppies having substantially lower respiration-specific N metabolism (Figure 1C; Figure 2C), which implies a higher metabolic C:N (less N metabolism per C metabolism). LP guppies live in environments that have C-rich but N-poor resources, whereas HP guppies subsist largely on lower C:N invertebrate foods (El-Sabaawi et al. 2012). The differences in excretion rates among populations used in this study may thus reflect adaptive shifts in metabolic fuels to more closely mirror the C:N of available resources. Notably, the differences on any treatment among populations of guppies occurs largely independent of the plastic effects of predation risk. Regardless of a guppy's evolutionary history with predation risk, our results indicate that it will induce adaptive, plastic changes in its metabolic C:N in response to predation risk, and this change will impact its role as a nutrient recycler.

Changes in tissue turnover time and implications for ecosystems

Fish can be important sources of limiting nutrients to aquatic ecosystems (McIntyre et al. 2008) and can alter ecosystem function (Knoll et al. 2009). Variation in excretion rates within species is substantial and largely unexplained (Torres and Vanni 2007) but important to ecological dynamics (Jeyasingh et al. 2014). Here, we demonstrate that predation risk reduces N excretion by an individual guppy by 25% and substantially increases the retention time of N in guppy tissues. Extending these results to field conditions would imply that the mere presence of predators can reduce N excretion by guppies, with potential effects on ecosystems (Bassar et al. 2012), slowing the rate at which nutrients cycle through guppies. While predators also have important effects on the size distribution and population abundance of guppies (Bassar et al.

2010), with implications for population-scale nutrient cycling (El-Sabaawi et al. 2015b), our results nevertheless show that the presence of predators can also transform the role of any individual guppy as a nutrient recycler. The influence of these effects in natural streams deserves further empirical attention, both within this system and in other systems where fish are intermediate consumers that contribute disproportionately to ecosystem biomass.

ACKNOWLEDGEMENTS

We are grateful to Amy R. McCune and Nelson G. Hairston, Jr. for comments that improved this manuscript. Thanks as well to Karen Tracy for help designing the fly-rearing protocol and procuring necessary materials, and to Kim Sparks for assistance with stable isotope analysis. This research was supported by NSF DEB 0623632, and NSF Doctoral Dissertation Improvement Grant, and financial assistance from the Cornell Biogeochemistry and Environmental Biocomplexity Small Grant, the Cornell Andrew W. Mellon Student Research Award, and the Cornell Sigma Xi Society. Christopher Dalton was supported by an NSF Pre-doctoral Fellowship during this research.

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TABLES

Table 1: Models for size-specific respiration rate (A), size-specific excretion rate (B), and respiration-specific excretion rate (C) of guppies measured only in water with no predator cues. "Pred Rearing" (or just "Pred" in interaction terms) reflects whether the guppy was reared in water with predator cues. "Population" reflects the population from which the guppy is descended. Each model also contains "family" as a random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

A. Models for Size Corrected Respiration					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pred Rearing	53.1	0.0	1.000	0.674	1.0
Population + Pred Rearing	56.0	2.9	0.235	0.158	4.3
Population + Pred Rearing + Population:Pred Rearing	57.0	3.9	0.140	0.094	7.2
No fixed effects	57.9	4.8	0.089	0.060	11.2
Population	61.1	8.0	0.019	0.013	53.4
B. Models for Size Corrected Excretion					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Population	48.9	0.0	1.000	0.723	1.0
Population + Pred Rearing	51.2	2.3	0.314	0.227	3.2
Population + Pred Rearing + Population:Pred Rearing	54.3	5.4	0.068	0.049	14.6
No fixed effects	97.2	48.4	0.000	0.000	>100
Pred Rearing	99.4	50.5	0.000	0.000	>100
C. Models for Respiration Corrected Excretion					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Population	10.5	0.0	1.000	0.686	1.0
Population + Pred Rearing	12.0	1.6	0.453	0.310	2.2
Population + Pred Rearing + Population:Pred Rearing	20.6	10.2	0.006	0.004	162.8
No fixed effects	71.4	60.9	0.000	0.000	>100
Pred Rearing	72.6	62.2	0.000	0.000	>100

Table 2: Best models for size-specific respiration rate (A), size-specific excretion rate (B), and respiration-specific excretion rate (C) of guppies reared with and without predation risk cues, measured in water both with and without predator cues. "Pred Rearing" (or just "Pred" in interaction terms) reflects whether the guppy was reared in water with predator cues. "Population" reflects the population from which the guppy is descended. "Cue" represents whether the incubation occurred in water with or without predation risk. Each model also contains "Fish ID" as a random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model. See Supplemental Tables 1-3 for comparison of all models.

A. Models for Size Corrected MO2 – Exp, 2					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pop+Pred Rear+Cue+Pred Rear×Cue	-408.4	0.0	1.000	0.181	1.0
Pop, Pred Rear, Cue, with all interactions	-407.8	0.6	0.725	0.132	1.4
Pop+Cue	-407.7	0.8	0.685	0.124	1.5
Pop+Pred Rear+Cue+Pop×Pred Rear+Pred Rear×Cue	-407.4	1.0	0.597	0.108	1.7
Pop+Pred Rear+Cue+Pop×Cue+Pred Rear×Cue	-406.9	1.5	0.466	0.084	2.1
Pop	-406.9	1.5	0.461	0.084	2.2
B. Models for Size Corrected Excretion – Exp. 2					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pop+Cue	646.6	0.0	1.000	0.177	1.0
Pop+Pred Rear+Cue+Pop×Pred Rear	646.9	0.3	0.864	0.153	1.2
Pop+Pred Rear+Cue	647.2	0.6	0.746	0.132	1.3
Pop +Cue+Pop×Cue	647.2	0.6	0.743	0.131	1.3
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue	647.7	1.1	0.577	0.102	1.7
Pop+Pred Rear+Cue+Pop×Cue	647.9	1.3	0.531	0.094	1.9
C. Models for Respiration Corrected Excretion - Exp. 2					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pop+Cue+Pop × Cue	568.2	0.0	1.000	0.464	1.0
Pop+Cue	570.1	1.9	0.384	0.178	2.6
Pop+Pred Rear+Cue+Pop×Cue	570.5	2.3	0.313	0.145	3.2
Pop + Pred Rear + Cue + Pop×Cue + Pred Rear×Cue	572.1	3.9	0.144	0.067	6.9
Pop + Pred Rear + Cue	572.3	4.2	0.125	0.058	8.0
Pop + Pred Rear + Cue + Pop×Pred Rear + Pop×Cue	573.8	5.7	0.059	0.027	16.9

Table 3: Models for the estimated turnover rate of N in guppies (*k*) based on isotopic labeling experiment. "Pred rearing" reflects the influence of being reared with predator cues during the experimental period (7-14 days), "Time" reflects the influence of the length of exposure to predator cues and labeled diets (7 or 14 days). The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Estimated Tissue Turnover Rate ('*k*')					
Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pred Rearing	-112.58	0.00	1.00	0.589	1.0
No Fixed Effects	-110.20	2.38	0.30	0.179	3.3
Time + Pred Rearing + Time	-109.71	2.86	0.24	0.141	4.2
Time	-107.65	4.93	0.09	0.050	11.8
Time + Pred Rearing + Time × Pred Rear	-107.26	5.32	0.07	0.041	14.3

FIGURES

Figure 1: Population and chronic predation risk effects on size-corrected respiration (A), size-corrected excretion (B), and respiration-corrected excretion (C) by guppies from one HP (blue triangles), one natural LP (red circles), and three introduced populations (light plum symbols) reared with and without predator cues. All incubations were conducted in predator-cue-free water. (A) Results as in Handelsman et al (2013). Populations tended to decrease respiration under predation risk, except for HP guppies. HP guppies have lower respiration rates in predator free environments than LP or introduced LP guppy populations. (B & C) Though HP guppies slightly elevated excretion under predation risk, all other populations did not alter excretion rates under chronic predation. LP guppies had lower excretion rates than HP, though introduced LP populations were not generally intermediate between HP and LP. Large symbols are treatment averages ± standard error. Small symbols are individual fish in each treatment. Some variation in x-axis added to facilitate visual comparison of groups.

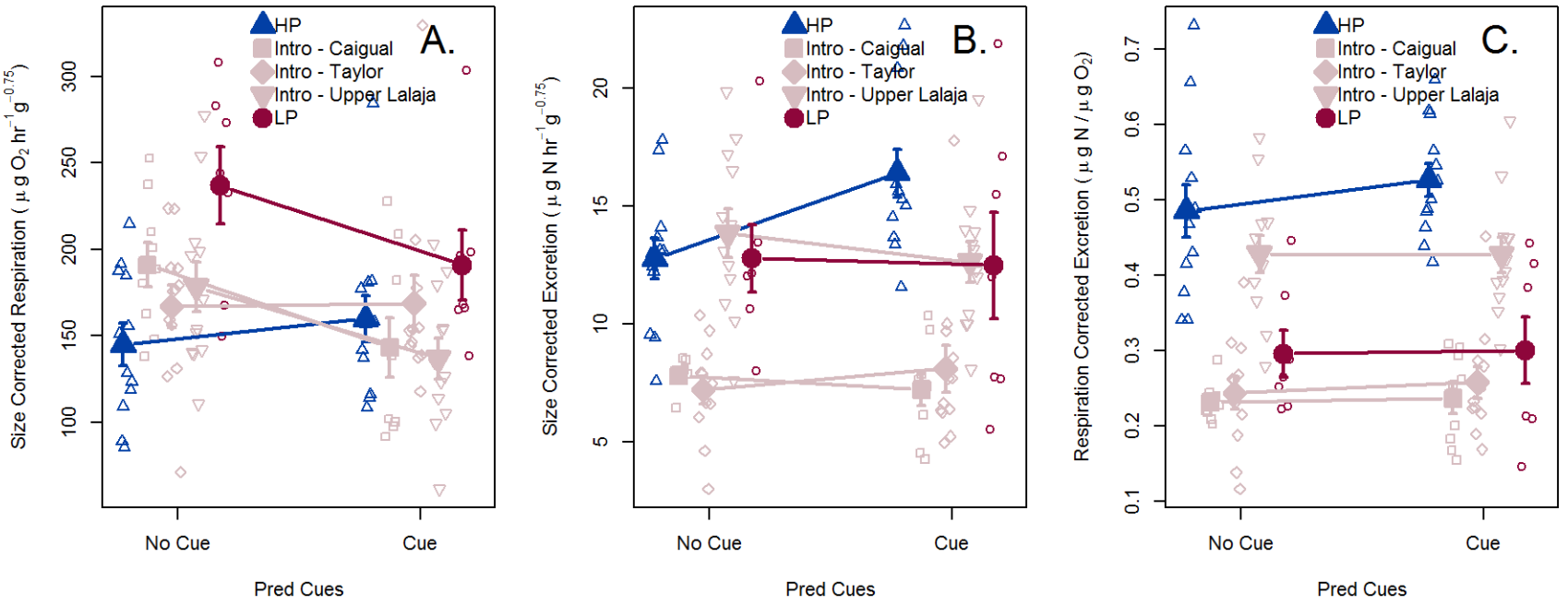


Figure 2: Population, chronic predation risk, and short-term predation risk effects on size-corrected respiration (A), size-corrected excretion (B), and respiration-corrected excretion (C) by guppies from one HP (blue triangles), one natural LP (red circles), and one introduced LP population (light plum symbols) reared with (open symbols) and without (closed symbols) predator cues. All incubations were conducted in predator-cue-free water. (A) No consistent treatment effects were detected on respiration. LP guppy respiration was significantly higher than that of HP guppies. (B & C) Whether corrected for fish size or fish respiration rate, guppy excretion declined when guppies were incubated for 24 h in predator-cue water, while chronic predation risk had no consistent effects on guppy excretion. (C) As in B, except that HP has highest respiration-specific excretion, introduced LP guppies have intermediate excretion, and natural LP has lowest respiration specific excretion. Large symbols are treatment averages ± standard error. Small symbols are individual fish in each treatment. Some variation in x-axis added to facilitate visual comparison of groups.

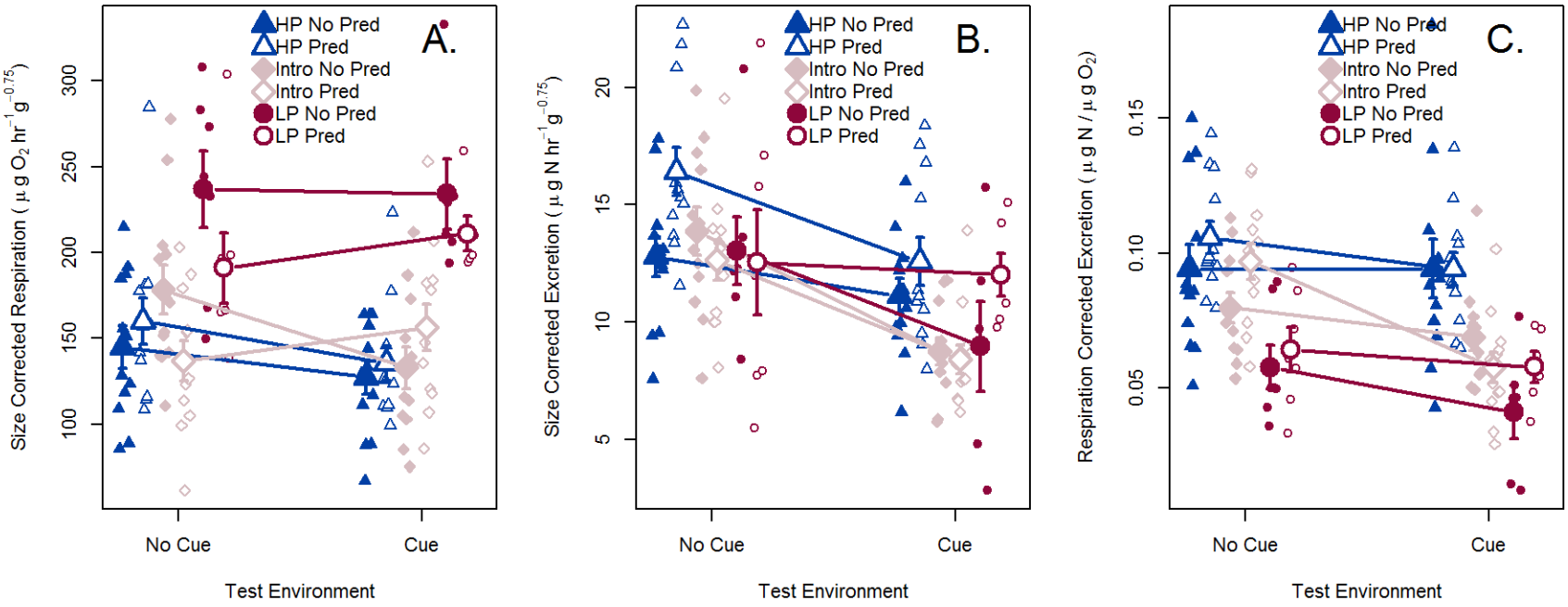


Figure 3: Effect sizes of short-term predation risk ("C"; light gray bars), chronic predation risk ("P"; medium gray bars), and both chronic and acute predation risk ("CP"; dark gray bars) on size corrected respiration (left), size corrected excretion (middle), and respiration corrected excretion (right). All treatments slightly reduced respiration, but only short-term predation risk reduced N excretion, whether corrected for size or for respiration rate. Bars are estimated mean effect sizes plus or minus standard errors (Nakagawa and Cuthill 2007).

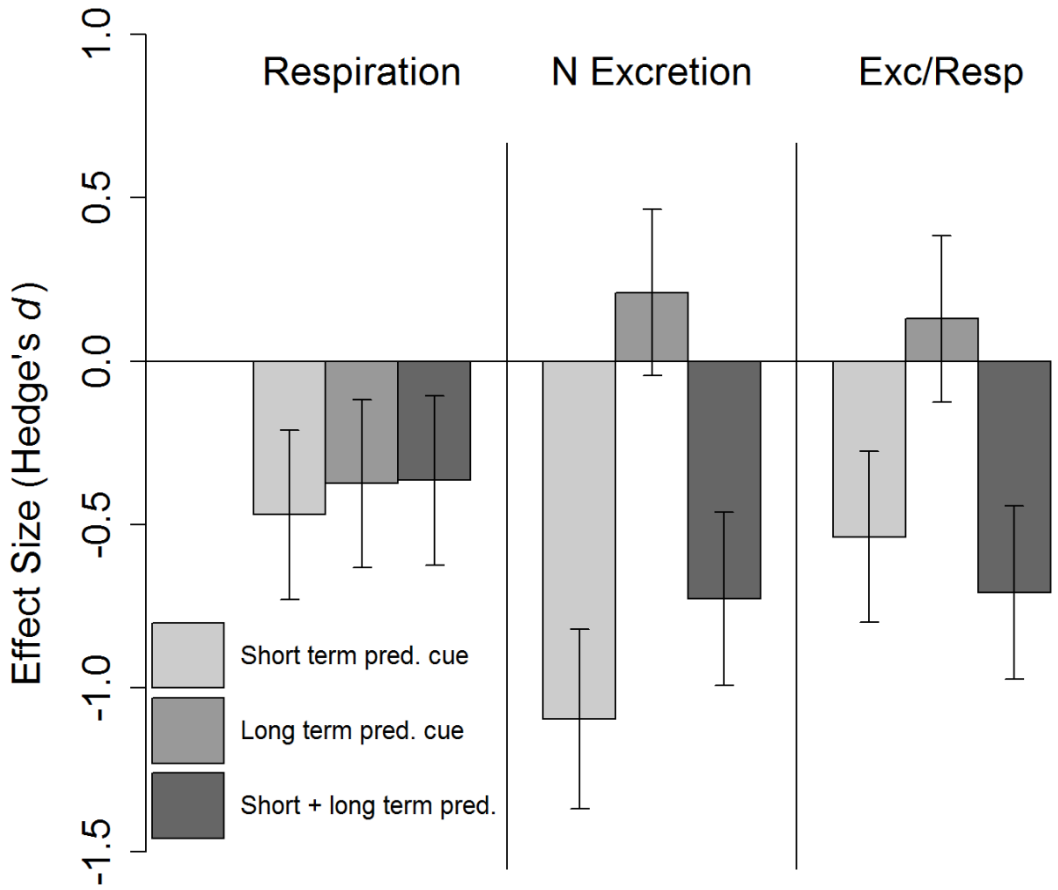
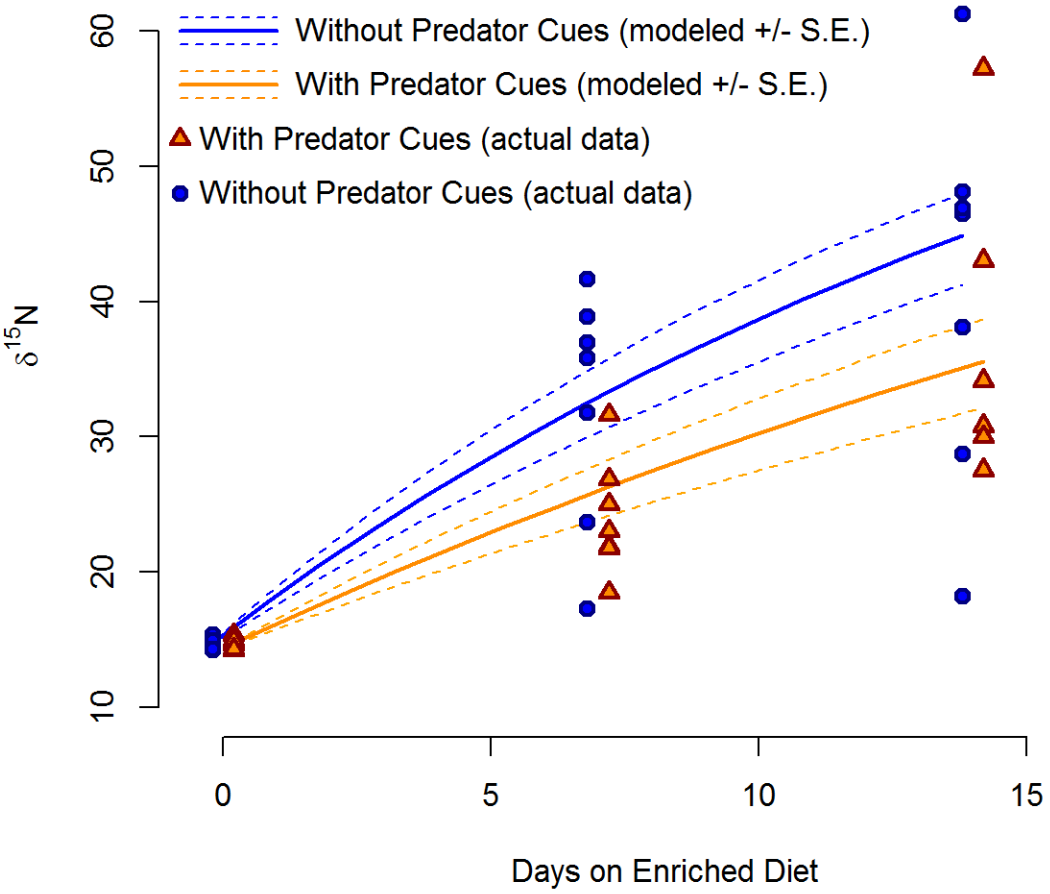
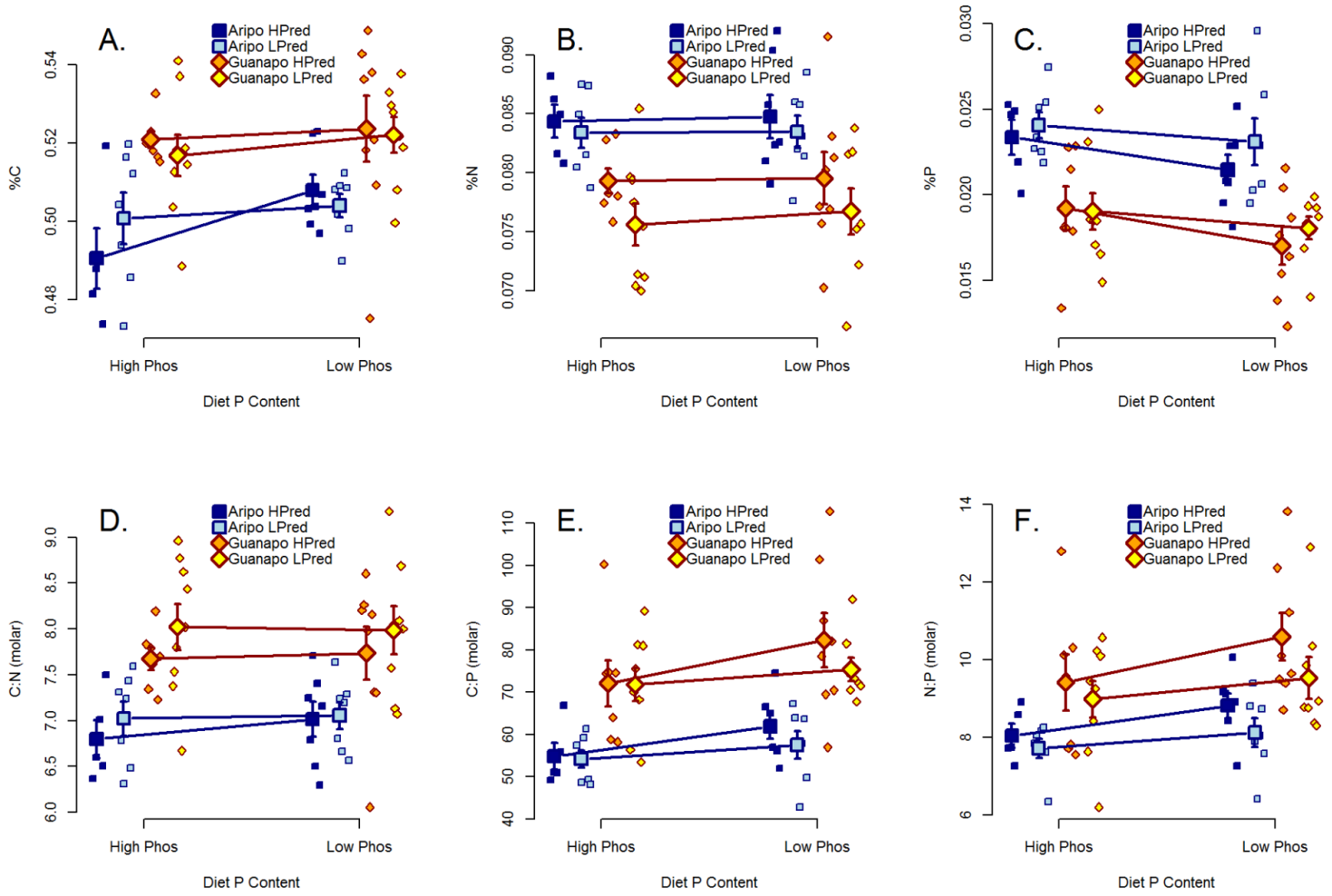


Figure 4: Modeled (lines ± standard error) and actual (points) estimates of $\delta^{15}N$ of guppies reared with (orange triangles, lines) and without (blue circles, lines) predator cues on an enriched diet for 7-14 days. Guppies were fed a diet enriched in ^{15}N during exposure to the predator treatment or control waters, and enrichment over the starting value reflects the extent of incorporation of consumed N during the treatment period. Guppies reared with predator-cue acquired the label more slowly than guppies reared without predator cue. Since none of the treatment groups changed weight during the experiment, these differences reflect predator-cue effects on the rate of protein turnover in guppies under varying predation risk environments. Modeled data are based entirely on treatment estimates of the decay parameter *k* (Equation 2) and associated standard errors.

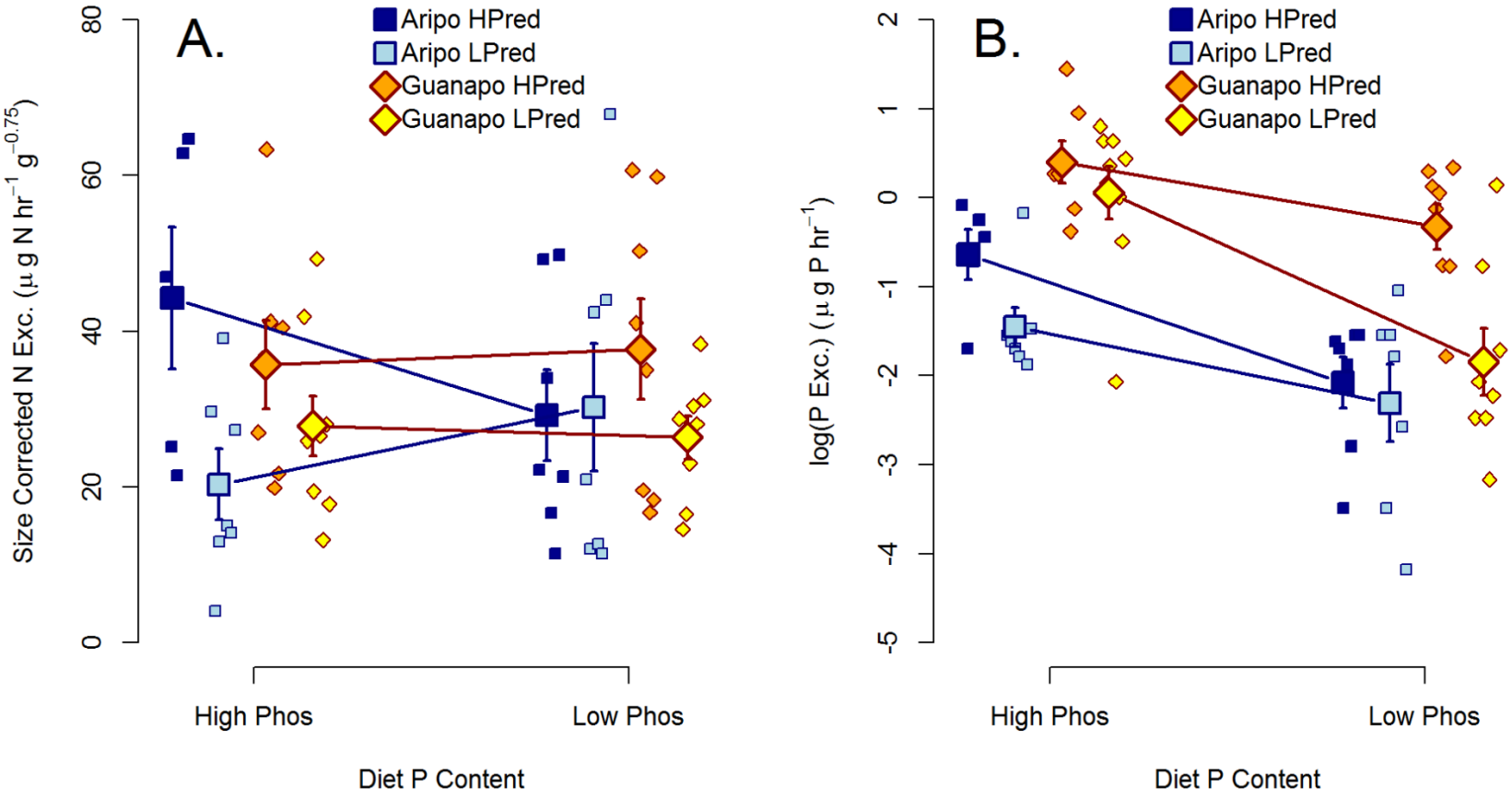


SUPPLEMENTAL MATERIALS – CHAPTER ONE

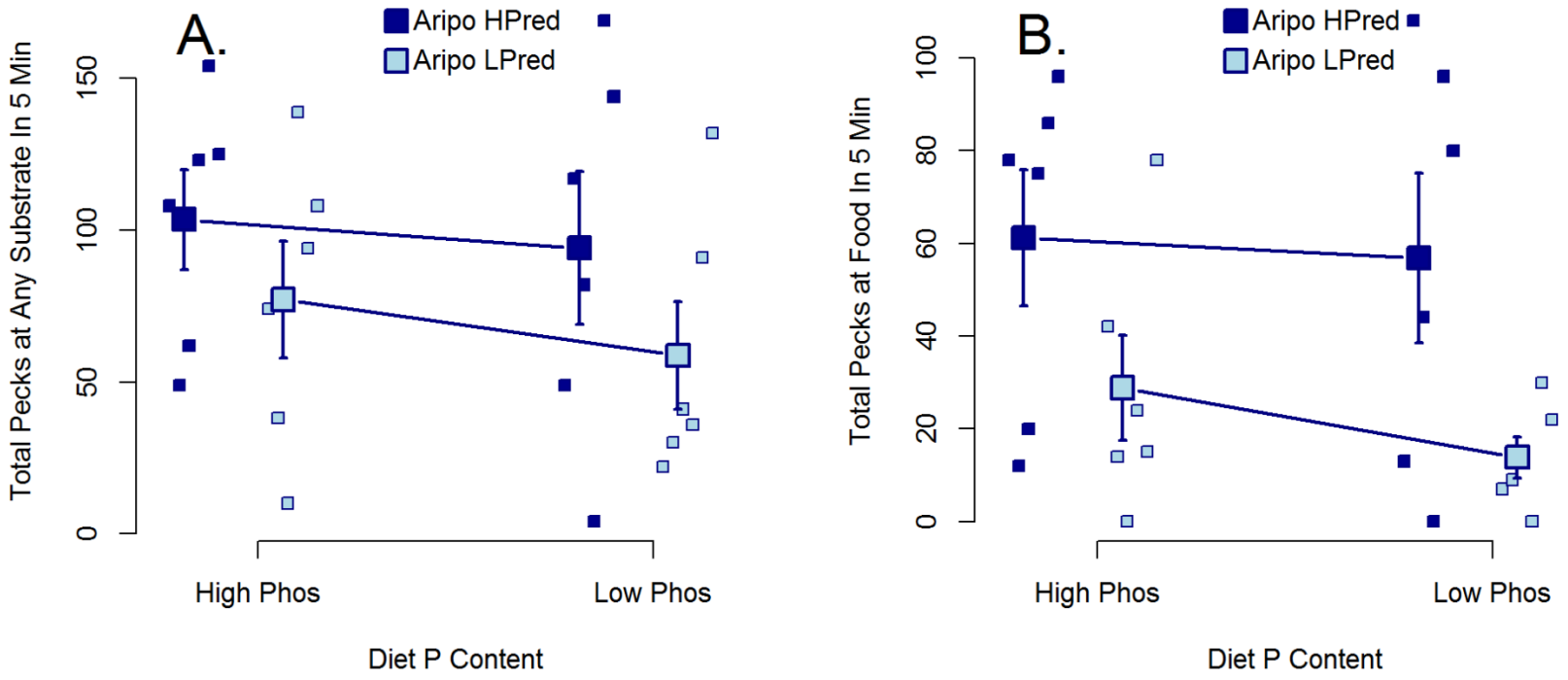
Supplemental Figure 1: Tissue stoichiometric variables (%C, %N, %P, C:N, C:P, N:P) by population and treatment. See supplemental Tables for significant effects on each variable. Large symbols are means and standard error bars. Small symbols are individual fish. Some variation in x-axis added to facilitate comparison of individual data points.



Supplemental Figure 2: Excretion stoichiometric variables (Size-corrected N Excretion and log-transformed P Excretion) by population and treatment. See Supplemental Tables 8-9 for significant effects on each variable. Large symbols are means and standard error bars. Small symbols are individual fish. Some variation in x-axis added to facilitate comparison of individual data points.



Supplemental Figure 3: Total Pecks at any substrate immediately after a feeding (A) and total pecks at high nutrient food immediately after a feeding (B). HPred guppies (dark squares) peck significantly more at food during a five minute test period than do LPred (light blue square; B), but the total number of foraging pecks (A) is not different, suggesting HPred guppies are feeding more selectively on high quality food items. Large symbols are means with standard error arrows. Small symbols are individual data points.



Supplemental Table 1: Diet formulation for high and low phosphorus diets, modified from Shim and Ho (1989). Total diet %P was 0.26% for the Low P and 0.81% for the High P diet. The total P content of the low and high P diets is below and above, respectively, the estimate of P limitation derived by Shim & Ho for domestic guppies. Sieved sand was added to the diet as an inert replacement, to keep the percent of all other diet items in the diet the same despite adding additional phosphorus to the high phosphorus diet.

	Low P Diet	High P Diet	
Ingredient	Amount added (g/ kg diet)	Amount added (g/ kg diet)	Ingredient P Content (g / kg item)
Vitamin Free Casein	351.2	351.2	7.5
Starch	150.5	150.5	-
Dextrin	141.6	141.6	-
Gelatin	78.1	78.1	0.2
Vegetable oil	50.0	50.0	-
Fish oil	50.1	50.1	-
Cellulose - FCC	10.0	10.0	-
Chlorine Chloride	5.0	5.0	-
Ascorbic Acid	1.2	1.2	-
DL-methionine	2.0	2.0	-
L-tryptophan	1.0	1.0	-
Betaine	10.0	10.0	-
Thiamine	0.0	0.0	-
Vitamin Premix	5.0	5.0	-
Dibasic Calcium Phosphate	0.0	30.1	182.1
Sieved sand	42.1	12.0	-
Mineral Mixture	102.0	102.0	-

The composition of the mineral mix was as below, all values in g / kg of mineral mix:

$MgSO_4$: 30.0; $FeSO_4 \times 7H_20$: 5.0; $NaHCO_3$: 88.8; $ZnCO_3$: 1.5; $CuSO_4 \times 5H_20$: 0.3; $MnSO_4 \times H_20$: 3.5; KIO_3: 0.1; $CoCL_2 \times 6H_20$: 0.02; $Na_2MoO_4 \times 2H_20$: 0.14; Alpha-cellulose: 727.9; $CaCO_3$: 142.8

Supplemental Table 2: Models for treatment and population effects on tissue %C. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue C	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv+Diet	-317.1	0.0	1.00	0.32	1.0
Riv	-316.6	0.6	0.76	0.24	1.3
Diet+Riv+Diet×Riv	-315.1	2.0	0.37	0.12	2.7
Riv+Pred+Diet	-314.6	2.5	0.29	0.09	3.5
Riv+Pred	-314.2	2.9	0.24	0.08	4.2
Diet+Pred+Riv+Diet×Riv	-312.5	4.6	0.10	0.03	9.9
Diet+Pred+Riv+Pred×Riv	-312.5	4.6	0.10	0.03	10.2
Diet+Pred+Riv+Diet×Pred	-312.4	4.7	0.10	0.03	10.5
Riv+Pred+Riv×Pred	-312.1	5.0	0.08	0.03	12.2
Diet+Pred+Riv+Diet×Riv+Pred×Riv	-310.3	6.8	0.03	0.01	29.8
Diet+Pred+Riv+Diet×Pred+Diet×Riv	-310.2	6.9	0.03	0.01	31.5
Diet+Pred+Riv+Diet×Pred+Pred×Riv	-310.2	6.9	0.03	0.01	32.1
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	-308.0	9.2	0.01	0.00	98.2
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	-306.3	10.9	0.00	0.00	>100
No Fixed Effects	-301.5	15.6	0.00	0.00	>100
Diet	-300.6	16.5	0.00	0.00	>100
Pred	-299.2	17.9	0.00	0.00	>100
Pred+Diet	-298.2	18.9	0.00	0.00	>100
Diet+Pred+Diet×Pred	-295.9	21.2	0.00	0.00	>100

Supplemental Table 3: Models for treatment and population effects on tissue %N. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue N	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv+Pred	-454.1	0.0	1.00	0.34	1.0
Riv	-453.0	1.2	0.56	0.19	1.8
Riv+Pred+Riv×Pred	-452.5	1.6	0.45	0.15	2.2
Riv+Pred+Diet	-451.8	2.3	0.31	0.10	3.2
Riv+Diet	-450.8	3.3	0.19	0.06	5.3
Diet+Pred+Riv+Pred×Riv	-450.1	4.0	0.13	0.04	7.5
Diet+Pred+Riv+Diet×Riv	-449.3	4.9	0.09	0.03	11.4
Diet+Pred+Riv+Diet×Pred	-449.2	4.9	0.09	0.03	11.7
Diet+Riv+Diet×Riv	-448.4	5.8	0.06	0.02	17.9
Diet+Pred+Riv+Diet×Riv+Pred×Riv	-447.4	6.7	0.04	0.01	28.3
Diet+Pred+Riv+Diet×Pred+Pred×Riv	-447.4	6.7	0.03	0.01	28.7
Diet+Pred+Riv+Diet×Pred+Diet×Riv	-446.6	7.5	0.02	0.01	43.3
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	-444.6	9.5	0.01	0.00	>100
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	-441.8	12.4	0.00	0.00	>100
No Fixed Effects	-437.0	17.1	0.00	0.00	>100
Pred	-436.4	17.7	0.00	0.00	>100
Diet	-435.0	19.2	0.00	0.00	>100
Pred+Diet	-434.2	20.0	0.00	0.00	>100
Diet+Pred+Diet×Pred	-431.7	22.4	0.00	0.00	>100

Supplemental Table 4: Models for treatment and population effects on tissue %P. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue P	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv+Diet	-511.6	0.0	1.00	0.32	1.0
Riv+Pred+Diet	-510.3	1.3	0.53	0.17	1.9
Riv	-509.3	2.2	0.33	0.11	3.0
Diet+Riv+Diet×Riv	-509.1	2.5	0.29	0.09	3.5
Riv+Pred	-508.4	3.2	0.21	0.07	4.8
Diet+Pred+Riv+Diet×Pred	-508.4	3.2	0.20	0.06	5.0
Diet+Pred+Riv+Pred×Riv	-508.0	3.5	0.17	0.05	5.9
Diet+Pred+Riv+Diet×Riv	-507.7	3.9	0.15	0.05	6.9
Riv+Pred+Riv×Pred	-506.2	5.3	0.07	0.02	14.4
Diet+Pred+Riv+Diet×Pred+Pred×Riv	-505.9	5.6	0.06	0.02	16.6
Diet+Pred+Riv+Diet×Pred+Diet×Riv	-505.7	5.9	0.05	0.02	19.1
Diet+Pred+Riv+Diet×Riv+Pred×Riv	-505.3	6.2	0.04	0.01	22.5
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	-503.1	8.4	0.01	0.00	67.2
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	-500.2	11.3	0.00	0.00	>100
No Fixed Effects	-485.6	26.0	0.00	0.00	>100
Diet	-484.7	26.8	0.00	0.00	>100
Pred	-484.2	27.4	0.00	0.00	>100
Pred+Diet	-483.2	28.4	0.00	0.00	>100
Diet+Pred+Diet×Pred	-480.7	30.8	0.00	0.00	>100

Supplemental Table 5: Models for treatment and population effects on tissue C:N (molar). Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue C:N	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv	110.0	0.0	1.00	0.32	1.0
Riv+Pred	110.4	0.4	0.83	0.27	1.2
Riv+Diet	112.3	2.3	0.31	0.10	3.2
Riv+Pred+Riv×Pred	112.5	2.5	0.29	0.09	3.5
Riv+Pred+Diet	112.7	2.7	0.26	0.08	3.9
Diet+Riv+Diet×Riv	114.7	4.7	0.10	0.03	10.3
Diet+Pred+Riv+Pred×Riv	114.9	4.9	0.09	0.03	11.7
Diet+Pred+Riv+Diet×Pred	115.1	5.1	0.08	0.03	12.6
Diet+Pred+Riv+Diet×Riv	115.1	5.1	0.08	0.02	13.0
Diet+Pred+Riv+Diet×Pred+Pred×Riv	117.4	7.4	0.02	0.01	40.8
Diet+Pred+Riv+Diet×Riv+Pred×Riv	117.5	7.5	0.02	0.01	42.3
Diet+Pred+Riv+Diet×Pred+Diet×Riv	117.6	7.6	0.02	0.01	44.6
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	120.1	10.1	0.01	0.00	>100
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	123.0	13.0	0.00	0.00	>100
No Fixed Effects	130.5	20.5	0.00	0.00	>100
Pred	131.9	21.9	0.00	0.00	>100
Diet	132.8	22.8	0.00	0.00	>100
Pred+Diet	134.3	24.3	0.00	0.00	>100
Diet+Pred+Diet×Pred	136.7	26.7	0.00	0.00	>100

Supplemental Table 6: Models for treatment and population effects on tissue C:P (molar). Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue C:P	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv+Diet	449.3	0.0	1.00	0.31	1.0
Riv+Pred+Diet	450.4	1.2	0.56	0.17	1.8
Riv	451.3	2.0	0.37	0.11	2.7
Diet+Riv+Diet×Riv	451.7	2.4	0.30	0.09	3.3
Diet+Pred+Riv+Diet×Pred	452.1	2.8	0.24	0.07	4.1
Riv+Pred	452.3	3.0	0.22	0.07	4.6
Diet+Pred+Riv+Diet×Riv	452.9	3.7	0.16	0.05	6.3
Diet+Pred+Riv+Pred×Riv	453.0	3.7	0.16	0.05	6.4
Diet+Pred+Riv+Diet×Pred+Diet×Riv	454.7	5.5	0.07	0.02	15.3
Diet+Pred+Riv+Diet×Pred+Pred×Riv	454.7	5.5	0.06	0.02	15.5
Riv+Pred+Riv×Pred	454.8	5.5	0.06	0.02	15.7
Diet+Pred+Riv+Diet×Riv+Pred×Riv	455.6	6.3	0.04	0.01	23.8
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	457.5	8.2	0.02	0.01	60.9
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	460.3	11.1	0.00	0.00	>100
No Fixed Effects	474.8	25.6	0.00	0.00	>100
Diet	475.6	26.4	0.00	0.00	>100
Pred	476.4	27.2	0.00	0.00	>100
Pred+Diet	477.4	28.1	0.00	0.00	>100
Diet+Pred+Diet×Pred	479.8	30.5	0.00	0.00	>100

Supplemental Table 7: Models for treatment and population effects on tissue N:P (molar). Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue N:P	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv+Pred+Diet	204.3	0.0	1.00	0.28	1.0
Riv+Diet	205.5	1.2	0.54	0.15	1.9
Diet+Pred+Riv+Diet×Pred	206.3	2.0	0.37	0.10	2.7
Riv+Pred	206.4	2.1	0.34	0.10	2.9
Diet+Pred+Riv+Diet×Riv	206.7	2.4	0.30	0.08	3.3
Diet+Pred+Riv+Pred×Riv	206.7	2.5	0.29	0.08	3.4
Diet+Riv+Diet×Riv	207.8	3.6	0.17	0.05	6.0
Riv	207.9	3.7	0.16	0.04	6.3
Riv+Pred+Riv×Pred	208.8	4.5	0.10	0.03	9.6
Diet+Pred+Riv+Diet×Pred+Diet×Riv	208.8	4.5	0.10	0.03	9.6
Diet+Pred+Riv+Diet×Pred+Pred×Riv	208.8	4.5	0.10	0.03	9.7
Diet+Pred+Riv+Diet×Riv+Pred×Riv	209.2	5.0	0.08	0.02	12.1
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	211.5	7.2	0.03	0.01	36.6
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	214.3	10.1	0.01	0.00	>100
Pred+Diet	218.4	14.2	0.00	0.00	>100
Pred	218.7	14.5	0.00	0.00	>100
Diet	218.8	14.5	0.00	0.00	>100
No Fixed Effects	219.3	15.0	0.00	0.00	>100
Diet+Pred+Diet×Pred	220.6	16.3	0.00	0.00	>100

Supplemental Table 8: Models for treatment and population effects on Size-corrected N excretion. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Size Corrected Excretion N	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Pred	484.3	0.0	1.00	0.36	1.0
No Fixed Effects	486.4	2.1	0.34	0.12	2.9
Riv+Pred	486.6	2.3	0.32	0.11	3.1
Pred+Diet	486.7	2.4	0.30	0.11	3.3
Diet+Pred+Diet×Pred	487.3	3.0	0.22	0.08	4.5
Riv	488.6	4.3	0.12	0.04	8.7
Diet	488.7	4.5	0.11	0.04	9.3
Riv+Pred+Diet	489.1	4.8	0.09	0.03	10.8
Riv+Pred+Riv×Pred	489.1	4.8	0.09	0.03	10.9
Diet+Pred+Riv+Diet×Pred	489.8	5.5	0.06	0.02	15.9
Riv+Diet	491.0	6.7	0.03	0.01	28.7
Diet+Pred+Riv+Diet×Riv	491.6	7.3	0.03	0.01	38.5
Diet+Pred+Riv+Pred×Riv	491.6	7.4	0.03	0.01	39.6
Diet+Pred+Riv+Diet×Pred+Diet×Riv	492.4	8.1	0.02	0.01	58.8
Diet+Pred+Riv+Diet×Pred+Pred×Riv	492.5	8.2	0.02	0.01	60.8
Diet+Riv+Diet×Riv	493.5	9.2	0.01	0.00	98.5
Diet+Pred+Riv+Diet×Riv+Pred×Riv	494.3	10.0	0.01	0.00	>100
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	495.2	10.9	0.00	0.00	>100
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	495.4	11.2	0.00	0.00	>100

Supplemental Table 9: Models for treatment and population effects on P excretion. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Excretion P	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv+Pred+Diet	155.9	0.0	1.00	0.37	1.0
Diet+Pred+Riv+Pred×Riv	157.5	1.6	0.44	0.16	2.3
Diet+Pred+Riv+Diet×Pred	157.7	1.9	0.39	0.14	2.5
Diet+Pred+Riv+Diet×Riv	158.3	2.4	0.30	0.11	3.3
Diet+Pred+Riv+Diet×Pred+Pred×Riv	159.4	3.5	0.17	0.06	5.8
Diet+Pred+Riv+Diet×Riv+Pred×Riv	159.9	4.1	0.13	0.05	7.6
Diet+Pred+Riv+Diet×Pred+Diet×Riv	160.2	4.4	0.11	0.04	8.8
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv+P×D×R	160.5	4.7	0.10	0.04	10.3
Diet+Pred+Riv+Diet×Pred+Diet×Riv+Pred×Riv	161.9	6.1	0.05	0.02	20.6
Riv+Diet	163.3	7.5	0.02	0.01	42.0
Diet+Riv+Diet×Riv	165.6	9.8	0.01	0.00	>100
Pred+Diet	175.5	19.7	0.00	0.00	>100
Riv+Pred	176.7	20.8	0.00	0.00	>100
Diet+Pred+Diet×Pred	178.0	22.1	0.00	0.00	>100
Riv	178.4	22.5	0.00	0.00	>100
Riv+Pred+Riv×Pred	178.4	22.6	0.00	0.00	>100
Diet	178.7	22.8	0.00	0.00	>100
Pred	187.8	31.9	0.00	0.00	>100
No Fixed Effects	188.6	32.7	0.00	0.00	>100

Supplemental Table 10: Models for treatment and population effects on PC1. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for PC1 (Tissue C vs. Tissue N and P)	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Riv	178.8	0.0	1.00	0.48	1.0
Pred+Riv	180.9	2.1	0.34	0.17	2.9
Diet+Riv	181.2	2.4	0.30	0.15	3.3
Pred * Riv	183.4	4.6	0.10	0.05	10.0
Pred+Diet+Riv	183.4	4.6	0.10	0.05	10.1
Diet * Riv	183.4	4.7	0.10	0.05	10.2
Pred+Diet+Riv+Pred×Diet	185.5	6.7	0.04	0.02	28.4
Pred+Diet+Riv+Diet×Riv	185.8	7.0	0.03	0.01	32.8
Pred+Diet+Riv+Pred×Riv	186.0	7.2	0.03	0.01	36.7
Pred+Diet+Riv+Pred×Diet+Diet×Riv	187.9	9.1	0.01	0.00	97.0
Pred+Diet+Riv+Pred×Diet+Pred×Riv	188.2	9.4	0.01	0.00	>100
Pred+Diet+Riv+Pred×Riv+Diet×Riv	188.5	9.7	0.01	0.00	>100
Pred+Diet+Riv+Pred×Diet+Pred×Riv+Diet×Riv	190.8	12.0	0.00	0.00	>100
Pred+Diet+Riv+Pred×Diet+Pred×Riv+Diet×Riv+P×D×R	193.6	14.8	0.00	0.00	>100
No Fixed Effects	211.4	32.6	0.00	0.00	>100
Pred	213.6	34.8	0.00	0.00	>100
Diet	213.7	34.9	0.00	0.00	>100
Pred+Diet	216.0	37.2	0.00	0.00	>100
Pred * Diet	218.5	39.7	0.00	0.00	>100

Supplemental Table 11: Models for treatment and population effects on PC2. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for PC2 (N and P Excretion)	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Pred+Diet	162.5	0.0	1.00	0.33	1.0
Pred * Diet	163.5	1.0	0.62	0.20	1.6
Pred	164.5	2.0	0.37	0.12	2.7
Pred+Diet+Riv	165.0	2.5	0.29	0.09	3.5
Pred+Diet+Riv+Pred×Diet	166.1	3.6	0.17	0.06	5.9
Pred+Riv	166.9	4.4	0.11	0.04	8.9
Pred+Diet+Riv+Pred×Riv	167.5	5.0	0.08	0.03	11.9
Pred+Diet+Riv+Diet×Riv	167.6	5.0	0.08	0.03	12.3
Diet	167.9	5.4	0.07	0.02	14.6
No Fixed Effects	168.6	6.1	0.05	0.02	21.2
Pred+Diet+Riv+Pred×Diet+Pred×Riv	168.7	6.2	0.05	0.01	21.8
Pred+Diet+Riv+Pred×Diet+Diet×Riv	168.7	6.2	0.05	0.01	22.0
Pred * Riv	169.2	6.7	0.03	0.01	28.6
Pred+Diet+Riv+Pred×Diet+Pred×Riv+Diet×Riv+P×D×R	169.4	6.8	0.03	0.01	30.2
Pred+Diet+Riv+Pred×Riv+Diet×Riv	170.1	7.6	0.02	0.01	45.0
Diet+Riv	170.3	7.7	0.02	0.01	47.5
Riv	170.9	8.4	0.02	0.00	65.3
Pred+Diet+Riv+Pred×Diet+Pred×Riv+Diet×Riv	171.4	8.9	0.01	0.00	86.3
Diet * Riv	172.7	10.2	0.01	0.00	>100

Supplemental Table 12: Models for treatment and population effects on PC3. Pred represents a fixed effect corresponding to the ancestral predation environment (HPred or LPred), and Diet represents a fixed effect corresponding to the level of phosphorus in the food of each guppy (high or low). Riv refers to the river of origin of each guppy (Aripo or Guanapo). Each model also contains "tank" as a random effect. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for PC3 (Tissue and Excretion P)	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Diet	138.5	0.0	1.00	0.49	1.0
Pred+Diet	140.8	2.3	0.32	0.15	3.1
Diet+Riv	140.9	2.4	0.30	0.15	3.3
Pred * Diet	142.7	4.2	0.12	0.06	8.2
Diet * Riv	143.2	4.7	0.10	0.05	10.5
Pred+Diet+Riv	143.2	4.8	0.09	0.04	10.8
Pred+Diet+Riv+Pred×Diet	145.3	6.8	0.03	0.02	29.7
Pred+Diet+Riv+Pred×Riv	145.3	6.8	0.03	0.02	30.4
Pred+Diet+Riv+Diet×Riv	145.7	7.2	0.03	0.01	36.3
Pred+Diet+Riv+Pred×Diet+Pred×Riv	147.4	8.9	0.01	0.01	85.7
Pred+Diet+Riv+Pred×Diet+Diet×Riv	147.8	9.3	0.01	0.00	>100
Pred+Diet+Riv+Pred×Riv+Diet×Riv	147.8	9.3	0.01	0.00	>100
Pred+Diet+Riv+Pred×Diet+Pred×Riv+Diet×Riv	149.9	11.5	0.00	0.00	>100
Pred+Diet+Riv+Pred×Diet+Pred×Riv+Diet×Riv+P×D×R	152.8	14.3	0.00	0.00	>100
No Fixed Effects	154.9	16.4	0.00	0.00	>100
Pred	156.9	18.4	0.00	0.00	>100
Riv	157.2	18.7	0.00	0.00	>100
Pred+Riv	159.3	20.8	0.00	0.00	>100
Pred * Riv	161.3	22.8	0.00	0.00	>100

SUPPLEMENTAL MATERIALS – CHAPTER TWO

Models for Individual basal resources

Basal resources were most strongly affected by the openness of canopy above a given pool. Areal chlorophyll concentrations increased with canopy cover and were lower in the Guanapo than the Aripo (Supplemental Table 2). Adding predator presence did not improve model explanatory power (likelihood ratio test (LRT): df = 1, χ^2 = 0.004, p = 0.947) and removing canopy cover and river did significantly reduce model explanatory power (LRT, canopy cover: df = 1, χ^2 = 8.265, p = 0.004; river: df = 1, χ^2 = 5.309, p = 0.02). The best models for epilithon organic matter and FBOM included no fixed effects (Supplemental Tables 3 and 4) and adding in river, predator presence, or canopy cover did not significantly improve the model performance (LRT, epilithon: river: df = 1, χ^2 = 0.016, p = 0.900; predator presence: df = 1, χ^2 = 1.67, p = 0.197; canopy cover: df = 1, χ^2 = 0.123, p = 0.726; LRT, FBOM: river: df = 1, χ^2 = 0.158, p = 0.691 ; predator presence: df = 1A, χ^2 = 2.395, p = 0.122; canopy cover: df = 1, χ^2 = 0.696, p = 0.404).

The abundance of invertebrates was positively related to the presence of predators (estimate = 321.9 ± S.E. = 63.2). Removing this term significantly reduced model explanatory power (LRT: df = 1, χ^2 = 13.791, p < 0.001), and adding river or canopy cover did not significantly increase model explanatory power (LRT, river: df = 1, χ^2 = 0.048, p = 0.826; canopy cover: df = 1, χ^2 = 2.38, p = 0.258; Supplemental Table 5).

Models for individual diet items

Guppy consumption of invertebrates at a given pool was positively correlated with resource PC2 in that pool (Supplemental Table 6; slope estimate = 0.346 ± S.E. = 0.155). Guppy

consumption of detritus was best explained by a predation by river interaction (Supplemental Table 7). Guppy consumption of detritus was significantly lower in Aripo sites with predators than all other sites (estimate = -1.377 ± S.E. = 0.366). A model for detritus consumption with the addition of a negative relationship between resource PC2 and diet had substantial support (Supplemental Table 7), but removing this resource PC2 effect did not significantly reduce the model's explanatory power (df = 1, χ^2 = 1.537, p = 0.215).

The amount of empty area in a guppy's gut was explained by predation and river, with guppies at sites with predators having more empty space in their guts (estimate = 0.492 ± S.E. = 0.219) and guppies in the Aripo having more empty space in their guts (estimate = 0.631 ± S.E. = 0.216). A model with a predation × river interaction received considerable support (ΔAICc = 0.2), but removing this term did not significantly reduce model explanatory power (LRT, df = 1, χ^2 = 1.992, p = 0.158). Similarly, a model with resource PC2 (positive correlation, slope estimate = 0.121 ± S.E. = 0.089), predation, and river received strong support, but removing the resource PC2 term did not significantly reduce model explanatory power (LRT: df = 1, χ^2 = 2.174, p = 0.140) while removing the predation term marginally reduced explanatory power (LRT: df = 1, χ^2 = 3.275, p = 0.070) and removing the river term significantly reduced model explanatory power (LRT: df = 1, χ^2 = 9.279, p = 0.002).

Models for individual stoichiometric measures

Tissue carbon content was best explained by Diet PC1, River, and a Diet PC1 × River interaction (Supplemental Table 9). Tissue carbon content increased with Diet PC1 score (differentiating guts with invertebrates from guts with detritus; slope estimate = 0.646 ± 0.292, p = 0.054), was always higher in the Guanapo than the Aripo (estimate = 2.68 ± S.E. = 0.883) and

increased with Diet PC1 at a faster rate in the Guanapo (estimate = 2.399 ± 1.379). Neither predation environment (estimate = 0.665 ± S.E. = 1.036, p = 0.537) nor Diet PC2 (differentiating empty guts from invertebrate filled guts; slope estimate = 0.219 ± 0.988) were related to tissue C content. A model without the Diet PC1 × River term also received substantial support, but removing this term reduced the explanatory power of the model (LRT: df = 1, χ^2 = 3.902, p = 0.048).

Tissue nitrogen content was best explained by diet and river (Supplemental Table 10). Tissue nitrogen content increased with diet PC1 scores (slope estimate = 0.109 ± S.E. = 0.017, p < 0.001). Tissue nitrogen was not related to diet PC2 (slope estimate = 0.084 ± 0.106, p = 0.448). Models with a River × Diet PC1 interaction or a predation environment effect also received substantial support, but removing either of these terms did not significantly reduce the explanatory power of the model (LRT: interaction: df = 1, χ^2 = 0.449, p = 0.503; predation: df = 1, χ^2 = 0.414, p = 0.520).

Tissue phosphorus content was best explained by a model with Diet PC1, River, Predation, and a Diet PC1 × River interaction (Supplemental Table 11). Removing the Predation term, however, did not reduce the explanatory power of the model (LRT: df = 1, χ^2 = 2.364, p = 0.124) and removing the Diet PC1 × River interaction only marginally reduced the model explanatory power (LRT: df = 1, χ^2 = 3.175, p = 0.075). Tissue P was negatively related to Diet PC1 (guppies eating less detritus had lower tissue phosphorus) and was lower in the Guanapo. A model with Diet PC2 instead of Diet PC1 also received substantial support, and, again, tissue P content was negatively related to the amount of invertebrates in the gut (and positively related to the empty area of the gut).

Size independent nitrogen excretion was best explained by a model with only predation as a fixed effect (Supplemental Table 12). Removing the predation term only marginally reduced model explanatory power (df = 1, χ^2 = 3.145, p = 0.076) and adding a Diet PC2 term did not increase model explanatory power (df = 1, χ^2 = 1.244, p = 0.265). Nitrogen excretion was lower in sites that had predators than in sites without predators (estimate = -10.21 ± S.E. = 5.87).

Size independent phosphorus excretion was best explained by a model with predation and river as effects (Supplemental Table 13). Guppies in sites with predators excreted P at a slower rate (estimate = -0.694 ± S.E. = 0.268). A model with only predation as a fixed effect received substantial support, and removing the river term did not reduce model explanatory power (LRT: df = 1, χ^2 = 2.277, p = 0.131) while removing the predation term did significantly reduce model explanatory power (LRT: df = 1, χ^2 = 7.847, p = 0.005).

Supplemental Tables

Supplemental Table 1: Models for the amount of canopy cover at a given pool in two rivers in Trinidad. Models included fixed effects of the presence of predator fish ("Pred"), the river ("Riv"), and the interaction of these main effects. The best model included no fixed effects, and adding predation into the model did not significantly improve the model explanatory power (likelihood ratio test (LRT): df = 1, $\chi^2 = 0.907$, p = 0.636). All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for Canopy Cover	AICc	ΔAICc	w_i	w_i ratio
Pred+Riv+Pred × Riv	298.6	7.3	0.01	38.4
Pred + Riv	295.7	4.4	0.06	9.0
Riv	293.9	2.6	0.15	3.6
Pred	293.0	1.7	0.24	2.3
No fixed effects	**291.3**	**0.0**	**0.54**	**1.0**

Supplemental Table 2: Models for the amount of chlorophyll *a* (μg cm^{-2}) per area in each of 36 sampled pools in two streams in Trinidad. Models included bottom up effects (canopy cover, "Canopy"), top down effects (presence of *Crenicichla*, "Pred"), river (Guanapo or Aripo, "Riv"), and all possible two-way interactions among these terms. The best model included effects of both canopy cover and river. A model with a canopy cover × predation interaction received modest support, but removing this interaction term did not significantly reduce model explanatory power (LRT: df = 1, $\chi^2 = 1.401$, p = 0.237). AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for areal Chlorophyll *a*	AICc	ΔAICc	w_i	w_i ratio
Canopy+Riv	134.5	0.0	0.33	1.0
Canopy+Riv+Canopy:Riv	136.1	1.6	0.15	2.2
Canopy	137.1	2.5	0.09	3.6
Canopy+Riv+Pred+Canopy:Pred	137.4	2.9	0.08	4.3
Canopy+Riv+Pred	137.5	3.0	0.07	4.4
Canopy+Riv+Pred+Riv:Pred	138.3	3.7	0.05	6.5
Canopy+Riv+Pred+Canopy:Pred+Pred:Riv	138.3	3.8	0.05	6.5
Canopy+Riv+Pred+Canopy:Riv	139.3	4.8	0.03	10.8
Canopy+Riv+Pred+Canopy:Riv+Pred:Riv	139.4	4.9	0.03	11.4
Pred+Canopy+Canopy:Pred	139.6	5.1	0.03	12.7
Canopy+Pred	139.6	5.1	0.03	12.8

Supplemental Table 3: Models for the amount of ash free dry mass in the epilithon (g cm^{-2}) per area in each of 36 sampled pools in two streams in Trinidad. Models included bottom up effects (canopy cover, "Canopy"), top down effects (presence of *Crenicichla*, "Pred"), river (Guanapo or Aripo, "Riv"), and all possible two-way interactions among these terms. The best model included no fixed effects. Adding predation or canopy cover did not improve model explanatory power (LRT: predation: df = 1, $\chi^2 = 1.667$, p = 0.197; canopy cover: df = 1, $\chi^2 = 0.123$, p = 0.726). AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for areal Epilithon AFDM	AICc	ΔAICc	w_i	w_i ratio
No fixed effects.	-365.5	0.0	0.35	1.0
Pred	-364.6	0.9	0.22	1.6
Canopy	-363.1	2.5	0.10	3.4
Riv	-363.0	2.6	0.10	3.6
Riv+Pred	-362.0	3.5	0.06	5.7
Canopy+Pred	-361.9	3.6	0.06	6.1
Pred+Riv+Riv:Pred	-361.3	4.3	0.04	8.5
Canopy+Riv	-360.3	5.2	0.03	13.5
Pred+Canopy+Canopy:Pred	-359.2	6.4	0.01	24.3
Canopy+Riv+Pred	-359.1	6.4	0.01	24.5
Canopy+Riv+Pred+Riv:Pred	-358.5	7.1	0.01	34.2

Supplemental Table 4: Models for the amount of fine benthic organic matter (fine detritus) (g cm^{-2}) per area in each of 36 sampled pools in two streams in Trinidad. Models included bottom up effects (canopy cover, "Canopy"), top down effects (presence of *Crenicichla*, "Pred"), river (Guanapo or Aripo, "Riv"), and all possible two-way interactions among these terms. The best model included no fixed effects. Adding predation or canopy cover did not improve model explanatory power (LRT: predation: df = 1, $\chi^2 = 2.361$, p = 0.124; canopy cover: df = 1, $\chi^2 = 0.684$, p = 0.408). AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for Detritus Concentration	AICc	ΔAICc	w_i	w_i ratio
1	-143.6	0.0	0.25	1.0
Pred	-143.3	0.2	0.23	1.1
Canopy	-141.7	1.9	0.10	2.6
Pred+Canopy+Canopy:Pred	-141.3	2.2	0.08	3.0
Riv	-141.1	2.4	0.08	3.3
Canopy+Pred	-140.9	2.6	0.07	3.7
Riv+Pred	-140.6	3.0	0.06	4.4
Pred+Riv+Riv:Pred	-139.3	4.2	0.03	8.3
Canopy+Riv	-139.0	4.5	0.03	9.7
Canopy+Riv+Pred+Canopy:Pred	-138.1	5.4	0.02	15.0

Canopy+Riv+Pred	-138.0	5.6	0.02	16.5

Supplemental Table 5: Models for the density of benthic invertebrates (individuals m^{-2}) in each of 36 sampled pools in two streams in Trinidad. Models included bottom up effects (canopy cover, "Canopy"), top down effects (presence of *Crenicichla*, "Pred"), river (Guanapo or Aripo, "Riv"), and all possible two-way interactions among these terms. The best model included only an effect of predation risk. A model with both predation risk and canopy cover received some support, but removing canopy cover did not significantly reduce model explanatory power (LRT: df = 1, $\chi^2 = 0.969$, p = 0.325). AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for Invertebrate Density	AICc	ΔAICc	w_i	w_i ratio
Pred	440.2	0.0	0.47	1.0
Canopy+Pred	441.6	1.4	0.23	2.0
Riv+Pred	443.0	2.7	0.12	3.9
Canopy+Riv+Pred	444.6	4.4	0.05	8.8
Pred+Canopy+Canopy:Pred	444.6	4.4	0.05	8.9
Pred+Riv+Riv:Pred	445.5	5.3	0.03	14.2
Canopy+Riv+Pred+Riv:Pred	447.3	7.1	0.01	34.4
Canopy+Riv+Pred+Canopy:Riv	447.7	7.4	0.01	41.4
Canopy+Riv+Pred+Canopy:Pred	447.8	7.5	0.01	43.4
Canopy+Riv+Pred+Canopy:Riv+Pred:Riv	450.6	10.3	0.00	176.0
Canopy+Riv+Pred+Canopy:Pred+Pred:Riv	450.7	10.5	0.00	191.8

Supplemental Table 6: Models for the diet invertebrate content in each of 159 guppy diets from 36 sampled pools in two streams in Trinidad. Models included bottom up effects of two resource principle component axes (see Table 2, Figure 1, "RPC1" and "RPC2"), top down effects (presence of *Crenicichla*, "P"), river (Guanapo or Aripo, "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for Invertebrate Consumption	AICc	ΔAICc	w_i	w_i ratio
RPC2	169.6	0.0	0.19	1.0
P + RPC2 + RPC2:P	171.2	1.7	0.08	2.3
RPC2 + R	171.5	1.9	0.07	2.6
RPC2 + P	171.7	2.1	0.07	2.9
RPC2 + R + RPC2:R	172.6	3.0	0.04	4.5
RPC2 + R + P + R:P	172.7	3.1	0.04	4.8
RPC2	169.6	0.0	0.19	1.0

Supplemental Table 7: Models for the diet detritus content in each of 159 guppy diets from 36 sampled pools in two streams in Trinidad. Models included bottom up effects of two resource principle component axes (see Table 2, Figure 1, "RPC1" and "RPC2"), top down effects (presence of *Crenicichla*, "P"), river (Guanapo or Aripo, "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for Detritus Consumption	AICc	ΔAICc	w_i	w_i ratio
P+R+R:P	237.0	0.0	0.33	3.0
RPC2+R+P+R:P	239.7	2.7	0.13	7.7
RPC1+R+P+R:P	239.9	2.9	0.14	7.3
RPC2+P	241.1	4.1	0.08	11.9
RPC2+R+P+RPC2:R+P:R	241.7	4.7	0.07	14.2
RPC2+R+RPC2:R	242.3	5.3	0.05	18.5
RPC1+R+P+RPC1:P+P:R	242.4	5.4	0.06	17.8

Supplemental Table 8: Models for the empty space in each of 159 guppy diets from 36 sampled pools in two streams in Trinidad. Models included bottom up effects of two resource principle component axes (see Table 2, Figure 1, "RPC1" and "RPC2"), top down effects (presence of *Crenicichla*, "P"), river (Guanapo or Aripo, "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of pools in each of six widely spaced sites at each river.

Models for Empty Gut Space	AICc	ΔAICc	w_i	w_i ratio
R+P	245.1	0.0	0.16	1.0
RPC2+R+P+RPC2:R	246.3	1.2	0.09	1.8
P+R+R:P	247.0	1.9	0.06	2.6
RPC2+R+P	247.3	2.2	0.05	3.0
RPC1+R+P+RPC1:P	247.5	2.4	0.05	3.3
RPC2+R	247.6	2.5	0.05	3.4
R	247.8	2.7	0.04	3.8

Supplemental Table 9: Models for the tissue carbon content of 102 guppies from 12 sampled pools in two streams in Trinidad. Models included bottom up effects of two diet principle component axes (see Table 4, Figure 2, represented below as "DPC1" and "DPC2"), top down effects (presence of *Crenicichla*, represented as "P"), river (Guanapo or Aripo, represented as "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All

models also include "access" as a random effect to account for non-independence of guppies collected and measured in the same pool.

Models for Tissue Carbon	AICc	ΔAICc	w_i	w_i ratio
DPC1 + R + DPC1:R	420.8	0.0	0.29	1.0
DPC1 + R	422.4	1.6	0.13	2.3
DPC1 + R + P + DPC1:R	423.0	2.3	0.09	3.1
DPC1 + R + P + R:P	423.2	2.5	0.08	3.5
DPC1 + R + P	424.2	3.5	0.05	5.7
DPC1 + R + P + DPC1:P	424.3	3.5	0.05	5.8
DPC1 + R + P + DPC1:R + P:R	425.0	4.3	0.04	8.4

Supplemental Table 10: Models for the tissue nitrogen content of 102 guppies diets from 12 sampled pools in two streams in Trinidad. Models included bottom up effects of two diet principle component axes (see Table 4, Figure 2, represented below as "DPC1" and "DPC2"), top down effects (presence of *Crenicichla*, represented as "P"), river (Guanapo or Aripo, represented as "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of guppies collected and measured in the same pool.

Models for Tissue Nitrogen	AICc	ΔAICc	w_i	w_i ratio
DPC1 + R	42.4	0.0	0.39	1.0
DPC1 + R + DPC1:R	44.2	1.8	0.16	2.5
DPC1 + R + P	44.2	1.8	0.15	2.5
DPC1 + R + P + DPC1:R	45.5	3.1	0.08	4.8
DPC1 + R + P + R:P	45.9	3.5	0.07	5.8
DPC1 + R + P + DPC1:P	46.1	3.7	0.06	6.2
DPC1 + R + P + DPC1:R + DPC1:P	47.5	5.1	0.03	13.1

Supplemental Table 11: Models for the tissue phosphorus content of 102 guppies diets from 12 sampled pools in two streams in Trinidad. Models included bottom up effects of two diet principle component axes (see Table 4, Figure 2, represented below as "DPC1" and "DPC2"), top down effects (presence of *Crenicichla*, represented as "P"), river (Guanapo or Aripo, represented as "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of guppies collected and measured in the same pool.

Models for Tissue Phosphorus	AICc	ΔAICc	w_i	w_i ratio
DPC1 + R + P + DPC1:R	120.3	0.0	0.11	1.0
DPC1 + R + DPC1:R	120.3	0.1	0.10	1.0
Diet.PC2 + R + P + Diet.PC2:R	120.8	0.6	0.08	1.3
DPC1 + R + P + DPC1:R + DPC1:P	121.0	0.7	0.07	1.4
DPC1 + R	121.2	1.0	0.07	1.6

Diet.PC2 + R + P + Diet.PC2:R + P:R	121.6	1.3	0.06	1.9
R	121.6	1.4	0.05	2.0
R + P	122.2	1.9	0.04	2.6

Supplemental Table 12: Models for size independent nitrogen excretion of 102 guppies diets from 12 sampled pools in two streams in Trinidad. Models included bottom up effects of two diet principle component axes (see Table 4, Figure 2, represented below as "DPC1" and "DPC2"), top down effects (presence of *Crenicichla*, represented as "P"), river (Guanapo or Aripo, represented as "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of guppies collected and measured in the same pool.

Models for Nitrogen Excretion	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
P	863.9	0.0	0.16	1.0
DPC2 + P	864.9	1.0	0.10	1.6
No Fixed Effects	864.9	1.0	0.10	1.6
DPC2	865.7	1.8	0.07	2.4
Diet.PC1	866.0	2.1	0.06	2.8
R + P	866.1	2.2	0.05	3.0
Diet.PC1 + P	866.1	2.2	0.05	3.0
P	863.9	0.0	0.16	1.0

Supplemental Table 13: Models for size independent phosphorus excretion of 102 guppies diets from 12 sampled pools in two streams in Trinidad. Models included bottom up effects of two diet principle component axes (see Table 4, Figure 2, represented below as "DPC1" and "DPC2"), top down effects (presence of *Crenicichla*, represented as "P"), river (Guanapo or Aripo, represented as "R"), and all possible two-way interactions among these terms. AICc is corrected Akaike Information Criteria, ΔAICc is the difference in AIC score and the AIC score of the best model, w_i, or the Akaike weight, reflects the probability that the specified model is the best model, and the w_i ratio specifies how much more likely the best model is than the specified model. All models also include "access" as a random effect to account for non-independence of guppies collected and measured in the same pool.

Models for Phosphorus Excretion	**AICc**	**ΔAICc**	**w_i**	**w_i ratio**
R + P	254.0	0.0	0.15	1.0
P	254.1	0.1	0.15	1.0
DPC2 + R + P + DPC2:R	255.7	1.6	0.07	2.2
DPC2 + P	255.8	1.7	0.06	2.3
P + R + R:P	256.0	1.9	0.06	2.6
DPC1 + P	256.2	2.2	0.05	2.9

SUPPLEMENTAL MATERIALS – CHAPTER THREE

Supplemental Table 1: Model selection results for dry food (a), carbon (b) and nitrogen consumption (c) during the experiment. Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are denoted by italicized text.

AIC-score Model Comparison | **Likelihood Ratio Test Results**

a Dry Food Consumed

#	Model Terms	Log. Lik.	AIC	ΔAIC
1	Pred+Diet+Pred:Diet	22.8	-33.6	2.2
2	Pred+Diet	22.0	-34.1	1.7
3	**Pred**	**21.9**	**-35.8**	**0.0**
4	1	-19.3	44.6	80.4

Models Comp.	Term Lost	χ^2	p-value
1,2	Pred:Diet	1.5	0.22
2,3	Diet	0.29	0.59
3,4	**Pred**	**82.3**	**<0.001**

b Carbon Consumed

#	Model Terms	Log. Lik.	AIC	ΔAIC
1	Pred+Diet+Pred:Diet	22.8	-33.6	0.8
2	Pred+Diet	22.0	-34.1	0.3
3	**Pred**	**21.2**	**-34.4**	**0.0**
4	1	-19.4	44.8	79.2

Models Comp.	Term Lost	χ^2	p-value
1,2	Pred:Diet	1.5	0.22
2,3	Diet	1.66	0.20
3,4	**Pred**	**81.2**	**<0.001**

c Nitrogen Consumed

#	Model Terms	Log. Lik.	AIC	ΔAIC
1	Pred+Diet+Pred:Diet	22.8	-33.6	0.5
2	**Pred+Diet**	**22.0**	**-34.1**	**0.0**
3	Pred	14.8	-21.6	12.5
4	Diet	-19.2	46.5	80.5

Models Comp.	Term Lost	χ^2	p-value
1,2	Pred:Diet	1.5	0.22
2,3	**Diet**	**14.6**	**<0.001**
2,4	**Pred**	**82.5**	**<0.001**

Supplemental Table 2: Model selection results for N excretion 1 hr post feeding (a), 8 hr post-feeding (b), and daily (c). Excretion 1 and 8 hr post feeding have log-transformed weight as a covariate. Total daily excretion has log-transformed daily N consumption as a covariate. Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are italicized.

AIC-score Model Comparison

a N Excretion 1 hr post feeding

#	Model Terms	Log Lik.	AIC	ΔAIC
1	lnWt*Pred*Diet	-14.7	49.5	6.6
2	lnWt+Pred+Diet+lnWt:Pred+lnWt:Diet+Pred:Diet	-14.8	47.5	4.6
3	lnWt+Pred+Diet+lnWt:Diet+Pred:Diet	-15.1	46.2	3.3
4	lnWt+Pred+Diet+Pred:Diet	-15.2	44.4	1.6
5	**lnWt+Pred+Diet**	**-15.4**	**42.9**	**0.0**
6	lnWt+Pred	-23.4	56.8	13.9
7	lnWt+Diet	-19.5	49.0	6.2
8	Pred+Diet	-22.7	55.4	12.5

b N Excretion 8 hr post feeding

#	Model Terms	Log Lik.	AIC	ΔAIC
1	lnWt*Pred*Diet	18.6	-17.3	5.7
2	lnWt+Pred+Diet+lnWt:Pred+lnWt:Diet+Pred:Diet	18.6	-19.3	3.7
3	lnWt+Pred+Diet+lnWt:Diet+Pred:Diet	18.6	-21.2	1.7
4	**lnWt+Pred+Diet+lnWt:Diet**	**18.5**	**-23.0**	**0.0**
5	lnWt+Pred+Diet	16.1	-20.1	2.8
6	lnWt+Pred	6.5	-3.1	19.9
7	lnWt+Diet	10.1	-10.2	12.8
8	Pred+Diet	10.3	-10.6	12.4

c Daily N Excretion

#	Model Terms	Log Lik.	AIC	ΔAIC
1	**lnWt*Pred*Diet**	**23.9**	**-27.8**	**0.00**
2	lnWt+Pred+Diet+lnWt:Pred+lnWt:Diet+Pred:Diet	21.0	-24.0	3.78
3	lnWt+Pred+Diet+lnWt:Diet+Pred:Diet	19.4	-22.8	4.97
4	lnWt+Pred+Diet+lnWt:Diet	19.4	-24.8	2.97
5	lnWt+Pred+Diet	19.1	-26.3	1.52
6	lnWt+Pred	5.0	-0.1	27.71
7	lnWt+Diet	10.6	-11.2	16.60
8	Pred+Diet	7.0	-4.0	23.83

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	lnWt:Pred:Diet	0.03	0.86
2,3	lnWt:Pred	0.66	0.42
3,4	lnWt:Diet	0.24	0.62
4,5	Pred:Diet	0.45	0.50
5,6	**Diet**	**15.92**	**<0.0001**
5,7	**Pred**	**8.16**	**0.004**
5,8	**lnWt**	**14.48**	**0.000**

Models Compared	Term Removed	χ^2	p-value
1,2	lnWt:Pred:Diet	0.01	0.92
2,3	lnWt:Pred	0.02	0.88
3,4	Pred:Diet	0.28	0.6
4,5	**lnWt:Diet**	**4.83**	**0.03**
5,6	**Diet**	**19.04**	**<0.0001**
5,7	**Pred**	**11.9**	**0.001**
5,8	**lnWt**	**11.6**	**0.001**

Models Compared	Term Removed	χ^2	p-value
1,2	**lnWt:Pred:Diet**	**5.8**	**0.02**
2,3	lnWt:Pred	3.2	0.07
3,4	Pred:Diet	0.0	0.95
4,5	lnWt:Diet	0.5	0.46
5,6	**Diet**	**28.2**	**<0.001**
5,7	**Pred**	**17.1**	**<0.001**
5,8	**lnWt**	**24.3**	**<0.001**

Supplemental Table 3: Model selection results for NUE_C (a), NUE_N (b), and NUE_C:NUE_N (c). Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are denoted by italicized text.

AIC-score Model Comparison | **Likelihood Ratio Test Results**

a Carbon use efficiency

#	Model Terms	Log. Lik.	AIC	ΔAIC
1	Pred+Diet+Pred:Diet	163.0	-314.0	2.0
2	**Pred+Diet**	**163.0**	**-315.9**	**0.0**
3	Pred	153.2	-298.5	17.4
4	Diet	161.6	-315.1	0.8

Models Comp.	Term Lost	χ^2	p-value
1,2	Pred:Diet	0.0	0.22
2,3	**Diet**	**19.4**	**<0.001**
3,4	*Pred*	*2.81*	*0.09*

b N use efficiency

#	Model Terms	Log. Lik.	AIC	ΔAIC
1	Pred+Diet+Pred:Diet	152.1	-292.2	3.7
2	Pred+Diet	152.1	-294.2	1.7
3	**Pred**	**151.9**	**-295.8**	**0.0**
4	Diet	140.2	-272.3	23.5

Models Comp.	Term Lost	χ^2	p-value
1,2	Pred:Diet	0.0	0.87
2,3	Diet	0.31	0.58
3,4	**Pred**	**23.8**	**<0.001**

c NUE_C : NUE_N

#	Model Terms	Log. Lik.	AIC	ΔAIC
1	Pred+Diet+Pred:Diet	97.8	-183.6	0.4
2	**Pred+Diet**	**97.0**	**-184.0**	**0.0**
3	Pred	63.1	-118.2	65.8
4	Diet	73.8	-139.7	44.4

Models Comp.	Term Lost	χ^2	p-value
1,2	Pred:Diet	1.6	0.21
2,3	**Diet**	**67.8**	**<0.001**
2,4	**Pred**	**46.4**	**<0.001**

Supplemental Table 4: Model selection and comparison results for total tissue nutrient content as a function of log-transformed standard length for C (a), N (b), and C:N (c). Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are denoted by italicized text.

a. Tissue C

AIC-score Model Comparison

#	Model Terms	Log Lik.	AIC	ΔAIC
1	SL*Pred*Diet	54.7	-89.4	5.1
2	SL+Pred+Diet+SL:Pred+SL:Diet+Pred:Diet	53.7	-89.5	5.0
3	SL+Pred+Diet+SL:Pred+Pred:Diet	53.7	-91.5	3.0
4	SL+Pred+Diet+Pred:Diet	53.3	-92.7	1.8
5	SL+Pred+Diet	52.7	-93.4	1.1
6	**SL+Pred**	**52.2**	**-94.5**	**0.0**
7	SL	43.2	-78.4	16.1
8	1	-28.8	63.7	158.1

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	SL:Pred:Diet	1.9	0.17
2,3	SL:Diet	0.0	0.87
3,4	SL:Pred	0.8	0.38
4,5	Pred:Diet	1.3	0.26
5,6	Diet	0.9	0.33
6,7	**Pred**	**18.1**	**<0.001**
7,8	**SL**	**144.1**	**<0.0001**

b. Tissue N

AIC-score Model Comparison

#	Model Terms	Log Lik.	AIC	ΔAIC
1	SL*Pred*Diet	69.0	-118.1	7.8
2	SL+Pred+Diet+SL:Pred+SL:Diet+Pred:Diet	68.7	-119.3	6.6
3	SL+Pred+Diet+SL:Pred+Pred:Diet	68.1	-120.2	5.7
4	SL+Pred+Diet+Pred:Diet	68.1	-122.2	3.7
5	SL+Pred+Diet	67.5	-123.0	2.9
6	SL+Pred	67.3	-124.5	1.4
7	**SL**	**67.0**	**-125.9**	**0.0**
8	1	-16.5	39.0	165.0

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	SL:Pred:Diet	0.8	0.39
2,3	SL:Diet	1.1	0.29
3,4	SL:Pred	0.0	0.95
4,5	Pred:Diet	1.2	0.28
5,6	Diet	0.5	0.49
6,7	Pred	0.6	0.44
7,8	**SL**	**167.0**	**<0.0001**

c. Tissue C:N

AIC-score Model Comparison

#	Model Terms	Log Lik.	AIC	ΔAIC
1	SL*Pred*Diet	-31.3	82.6	5.3
2	SL+Pred+Diet+SL:Pred+SL:Diet+Pred:Diet	-31.6	81.1	3.8
3	SL+Pred+Diet+SL:Pred+Pred:Diet	-31.6	79.2	1.9
4	**SL+Pred+Diet+SL:Pred**	**-31.7**	**77.3**	**0.0**
5	SL+Pred+Diet	-34.1	80.3	3.0
6	SL+Pred	-37.0	83.9	6.6
7	SL+Diet	-47.2	104.4	27.1
8	Pred+Diet	-36.3	82.6	5.3

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	SL:Pred:Diet	0.5	0.48
2,3	SL:Diet	0.1	0.81
3,4	Pred:Diet	0.1	0.74
4,5	**SL:Pred**	**5**	**0.03**
5,6	**Diet**	**5.7**	**0.02**
5,7	**Pred**	**26.2**	**<0.0001**
5,8	**SL**	**4.4**	**0.04**

Supplemental Table 5: Model selection results for guppy length (a), weight (b), condition factor (c) and specific growth (d).

AIC-score Model Comparison

Likelihood Ratio Test Results

a Fish Length

#	Model Terms	Log Lik.	AIC	ΔAIC
1	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet+Age:Pred:Diet	259.8	-497.6	3.0
2	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet	259.8	-499.6	1.1
3	Age+Pred+Diet+Age:Pred+Pred:Diet	258.9	-499.9	0.8
4	**Age+Pred+Diet+Age:Pred**	**258.3**	**-500.6**	**0.0**
5	Age+Pred+Diet	247.8	-481.7	19.0
6	Age+Pred+Age:Pred	255.9	-497.7	2.9

Models Compared	Term Removed	χ^2	p-value
1,2	Age:Pred:Diet	0.1	0.78
2,3	Age:Diet	1.7	0.2
3,4	Pred:Diet	1.3	0.26
4,5	**Age:Pred**	**29.0**	**<0.001**
4,6	**Diet**	**16.0**	**<0.001**

b Fish Weight

#	Model Terms	Log Lik.	AIC	ΔAIC
1	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet+Age:Pred:Diet	85.9	-149.9	2.3
2	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet	85.7	-151.4	0.8
3	Age+Pred+Diet+Age:Pred+Pred:Diet	85.0	-152.0	0.2
4	**Age+Pred+Diet+Age:Pred**	**84.1**	**-152.2**	**0.00**
5	Age+Pred+Diet	69.2	-124.5	27.8
6	Age+Pred+Age:Pred	81.7	-149.4	2.8

Models Compared	Term Removed	χ^2	p-value
1,2	Age:Pred:Diet	0.4	0.51
2,3	Age:Diet	1.5	0.23
3,4	Pred:Diet	1.8	0.18
4,5	**Age:Pred**	**29.8**	**<0.001**
4,6	**Diet**	**4.8**	**0.03**

c Condition Factor

#	Model Terms	Log Lik.	AIC	ΔAIC
1	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet+Age:Pred:Diet	237.8	-453.5	5.8
2	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet	237.4	-454.8	4.5
3	Age+Pred+Diet+Age:Pred+Pred:Diet	237.4	-456.8	2.5
4	Age+Pred+Diet+Age:Pred	236.8	-457.6	1.7
5	**Age+Pred+Age:Pred**	**236.6**	**-459.3**	**0.0**
6	Age+Pred	234.3	-456.5	2.8

Models Compared	Term Removed	χ^2	p-value
1,2	Age:Pred:Diet	0.7	0.41
2,3	Age:Diet	0.1	0.83
3,4	Pred:Diet	1.1	0.29
4,5	Diet	0.4	0.55
5,6	**Age:Pred**	**4.8**	**0.03**

d Specific Growth

#	Model Terms	Log Lik.	AIC	ΔAIC
1	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet+Age:Pred:Diet	548.9	-1075.8	2.7
2	Age+Pred+Diet+Age:Pred+Age:Diet+Pred:Diet	548.8	-1077.5	0.9
3	**Age+Pred+Diet+Age:Pred+Pred:Diet**	**548.2**	**-1078.4**	**0.0**
4	Age+Pred+Diet+Age:Pred	546.8	-1077.5	0.9
5	Age+Pred+Diet	540.9	-1067.8	10.7
6	Age+Pred+Age:Pred	542.2	-1070.5	8.0

Models Compared	Term Removed	χ^2	p-value
1,2	Age:Diet:Pred	0.2	0.89
2,3	Age:Diet	1.1	0.30
3,4	*Pred:Diet*	*2.9*	*0.09*
4,5	**Age:Pred**	**11.8**	**<0.001**
4,6	**Diet**	**9.0**	**0.002**

Supplemental Table 6: Model selection and comparison results for reproductive tissue dry mass (log-transformed) as a function of log-transformed somatic dry weight and treatments. Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are denoted by italicized text.

AIC-score Model Comparison

#	Model Terms	Log Lik.	AIC	ΔAIC
1	DryWt*Pred*Diet	358.7	-697.5	3.4
2	DryWt+Pred+Diet+DryWt:Pred+DryWt:Diet+Pred:Diet	358.3	-698.7	2.2
3	DryWt+Pred+Diet+DryWt:Pred+Pred:Diet	356.7	-697.4	3.5
4	DryWt+Pred+Diet+Pred:Diet	356.7	-699.4	1.5
5	DryWt+Pred+Diet	355.6	-699.1	1.8
6	DryWt+Pred	355.2	-700.3	0.6
7	**DryWt**	**354.4**	**-700.9**	**0.0**
8	1	348.9	-691.8	9.0

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	DryWt:Pred:Diet	0.8	0.37
2,3	*DryWt:Diet*	*3.3*	*0.07*
3,4	DryWt:Pred	0.0	0.92
4,5	Pred:Diet	2.3	0.13
5,6	Diet	0.8	0.37
6,7	Pred	1.4	0.23
7,8	**DryWt**	**11.0**	**0.001**

Supplemental Table 7: Model selection and comparison results for reproductive tissue carbon (log-transformed) as a function of log-transformed somatic dry weight and treatments. Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are denoted by italicized text.

AIC-score Model Comparison

#	Model Terms	Log Lik.	AIC	ΔAIC
1	SomC*Pred*Diet	397.6	-775.3	2.4
2	SomC+Pred+Diet+SomC:Pred+SomC:Diet+Pred:Diet	397.3	-776.6	1.1
3	SomC+Pred+Diet+SomC:Pred+Pred:Diet	395.5	-774.9	2.7
4	SomC+Pred+Diet+Pred:Diet	395.5	-776.9	0.7
5	SomC+Pred+Diet	394.4	-776.8	0.9
6	**SomC+Pred**	**393.8**	**-777.7**	**0.0**
7	SomC	393.3	-776.5	1.1
8	1	387.7	-769.4	8.3

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	SomC:Pred:Diet	0.7	0.40
2,3	SomC:Diet	3.7	0.06
3,4	SomC:Pred	0.0	0.93
4,5	Pred:Diet	2.2	0.14
5,6	Diet	1.1	0.29
6,7	Pred	1.9	0.17
7,8	**SomC**	**10.3**	**0.001**

Supplemental Table 8: Model selection and comparison results for reproductive tissue nitrogen (log-transformed) as a function of log-transformed somatic dry weight and treatments. Best fit models and significant model terms are highlighted in bold. Marginally significant model terms are denoted by italicized text.

AIC-score Model Comparison

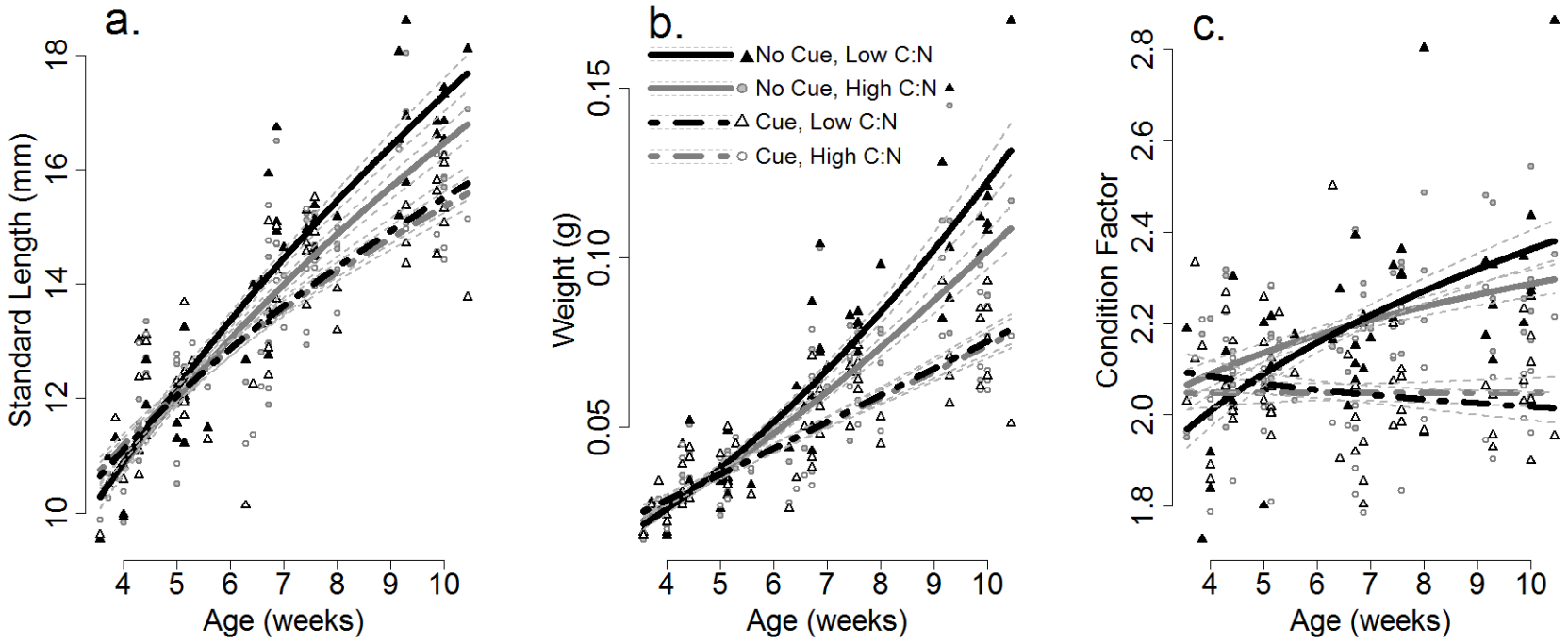
#	Model Terms	Log Lik.	AIC	ΔAIC
1	SomN*Pred*Diet	532.0	-1044.0	5.4
2	SomN+Pred+Diet+SomN:Pred+SomN:Diet+Pred:Diet	531.7	-1045.5	3.9
3	SomN+Pred+Diet+SomN:Pred+Pred:Diet	530.5	-1045.1	4.3
4	SomN+Pred+Diet+Pred:Diet	530.5	-1047.0	2.3
5	SomN+Pred+Diet	529.5	-1047.0	2.4
6	SomN+Pred	529.4	-1048.7	0.6
7	**SomN**	**528.7**	**-1049.3**	**0.0**
8	1	522.1	-1038.2	11.2

Likelihood Ratio Test Results

Models Compared	Term Removed	χ^2	p-value
1,2	SomN:Pred:Diet	0.5	0.48
2,3	SomN:Diet	2.4	0.12
3,4	SomN:Pred	0.1	0.78
4,5	Pred:Diet	2.0	0.15
5,6	Diet	0.2	0.62
6,7	Pred	1.4	0.24
7,8	**SomN**	**13.2**	**0.0002**

Supplemental Figure 1: Guppy length (a), weight (b) and condition factor (c) over the course of the experiment as a function of fish age for the high C:N diet (gray circles, lines), low C:N diet (black triangles, lines), no cue (solid lines, filled symbols) and cue (dashed lines, open symbols) treatments. Lines represent best-fit linear regressions (± model standard error, fine dashed lines) for each treatment group of log-transformed length, weight or condition factor as a function of log-transformed age. Sample size is sixteen for each treatment group.

Figure 1



SUPPLEMENTAL MATERIALS – CHAPTER FOUR

Supplemental Table 1: Models for treatment effect combinations on specific growth rate. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Specific Growth	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Pred+Food	-480.2	0.0	1.00	0.72	1.0
Pred+Food+Pred × Food	-478.3	1.9	0.39	0.28	2.6
Food	-466.4	13.8	0.00	0.00	>100
Pred	-431.8	48.4	0.00	0.00	>100
1	-427.8	52.4	0.00	0.00	>100

Supplemental Table 2: Models for treatment effect combinations on tissue dry weight to wet weight ratio. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Dry Wt:Wet Wt	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Pred+Food+Pred × Food	-393.8	0.0	1.00	0.98	1.0
Pred+Food	-386.4	7.4	0.02	0.02	40.9
Food	-377.1	16.7	0.00	0.00	>100
Pred	-348.3	45.5	0.00	0.00	>100
1	-345.4	48.4	0.00	0.00	>100

Supplemental Table 3: Models for treatment effect combinations on food consumption by each guppy during the experiment. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Food consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Food Consumption	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Pred+Food	105.6	0.0	1.00	0.74	1.0
Pred+Food+Pred×Food	107.7	2.1	0.35	0.26	2.9
Food	127.4	21.8	0.00	0.00	>100
Pred	171.1	65.5	0.00	0.00	>100
1	177.7	72.2	0.00	0.00	>100

Supplemental Table 4: Models for treatment effect combinations and measured food consumption on specific growth by each guppy during the experiment. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). *Cons* refers to the measured consumption of food during the experiment. Each model also contains "block" and "population" as random effects. Food consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Specific Growth	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Cons	-487.1	0.0	1.00	0.31	1.0
Cons+Food	-485.9	1.2	0.55	0.17	1.8
Cons+Pred+Food	-485.2	1.9	0.39	0.12	2.6
Cons+Pred	-484.8	2.3	0.31	0.10	3.2
Cons+Pred+Food+Cons×Pred	-484.7	2.4	0.30	0.09	3.4
Cons+Pred+Food+Pred×Food	-483.6	3.5	0.17	0.05	5.8
Cons+Pred+Food+Cons×Food	-483.3	3.8	0.15	0.05	6.5
Cons+Pred+Food+Cons×Pred+Pred×Food	-482.2	4.9	0.09	0.03	11.7
Cons+Pred+Food+Cons×Pred+Cons×Food	-482.0	5.1	0.08	0.02	12.9
Cons+Pred+Food+Cons×Food+Pred×Food	-481.1	6.0	0.05	0.02	20.5
Cons+Pred+Food+Cons×Pred+Cons×Food+P×F+C×P×F	-480.5	6.6	0.04	0.01	26.6
Pred+Food	-480.2	6.9	0.03	0.01	31.9
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food	-479.4	7.6	0.02	0.01	45.7
Food	-466.4	20.7	0.00	0.00	>100
Pred	-431.8	55.3	0.00	0.00	>100

Supplemental Table 5: Models for treatment effect combinations and measured food consumption on the final tissue dry weight : wet weight ratio at the end of the experiment. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). *Cons* refers to the measured consumption of food during the experiment. Each model also contains "block" and "population" as random effects. Food consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Total Tissue Dry Weight : Wet Weight	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Cons	-405.5	0.0	1.00	0.54	1.0
Cons+Pred	-403.1	2.4	0.30	0.16	3.3
Cons+Food	-403.1	2.4	0.30	0.16	3.4
Cons+Pred+Food	-400.6	4.9	0.09	0.05	11.7
Cons+Pred+Food+Pred×Food	-399.5	6.1	0.05	0.03	20.8
Cons+Pred+Food+Cons×Pred	-398.3	7.3	0.03	0.01	38.2
Cons+Pred+Food+Cons×Food+Pred×Food	-398.2	7.4	0.02	0.01	40.0
Cons+Pred+Food+Cons×Food	-398.1	7.4	0.02	0.01	40.9
Cons+Pred+Food+Cons×Pred+Pred×Food	-398.0	7.5	0.02	0.01	43.2
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food	-396.3	9.2	0.01	0.01	>100
Cons+Pred+Food+Cons×Pred+Cons×Food	-396.0	9.5	0.01	0.00	>100
Cons+Pred+Food+Cons×Pred+Cons×Food+P×F+C×P×F	-393.5	12.0	0.00	0.00	>100
Pred+Food	-386.4	19.2	0.00	0.00	>100
Food	-377.1	28.4	0.00	0.00	>100
Pred	-348.3	57.2	0.00	0.00	>100

Supplemental Table 6: Models for treatment effect combinations and fish length (log-transformed) on total tissue C content (also log-transformed) at the end of the experiment. *SL* represents standard length, which was a log-transformed and a covariate throughout this table. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Fish length was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Total Tissue C Stocks (log transformed)	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
SL+Pred+Food+SL×Pred	-105.8	0.0	1.00	0.22	1.0
SL+Pred+Food	-105.7	0.1	0.97	0.21	1.0
SL+Pred+Food+Pred×Food	-105.2	0.6	0.74	0.16	1.4
SL+Pred+Food+SL×Pred+Pred×Food	-104.4	1.3	0.52	0.11	1.9
SL+Pred+Food+SL×Food	-103.8	2.0	0.38	0.08	2.7
SL+Pred+Food+SL×Pred+SL×Food	-103.8	2.0	0.37	0.08	2.7
SL+Pred+Food+SL×Food+Pred×Food	-103.0	2.8	0.25	0.05	4.0
SL+Pred+Food+SL×Pred+SL×Food+Pred×Food	-102.2	3.6	0.17	0.04	5.9
SL+Food	-101.3	4.5	0.11	0.02	9.4
SL+Pred+Food+SL×Pred+SL×Food+Pred×Food+SL×P×F	-100.7	5.1	0.08	0.02	12.6
SL+Pred	-75.5	30.3	0.00	0.00	>100
SL	-74.6	31.2	0.00	0.00	>100
Pred+Food	-30.0	75.8	0.00	0.00	>100
Food	-19.9	85.9	0.00	0.00	>100
Pred	16.1	121.8	0.00	0.00	>100

Supplemental Table 7: Models for treatment effect combinations and fish length (log-transformed) on total tissue N content (also log-transformed) at the end of the experiment. *SL* represents standard length, which was a log-transformed and a covariate throughout this table. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Fish length was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Total Tissue N Stocks (log transformed)	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
SL+Food	-203.1	0.0	1.00	0.49	1.0
SL+Pred+Food	-200.7	2.4	0.30	0.15	3.3
SL+Pred+Food+SL×Food	-200.0	3.1	0.22	0.11	4.6
SL+Pred+Food+SL×Pred	-199.5	3.6	0.16	0.08	6.1
SL+Pred+Food+SL×Pred+SL×Food	-198.7	4.3	0.11	0.06	8.8
SL+Pred+Food+Pred×Food	-198.2	4.9	0.08	0.04	11.8
SL+Pred+Food+SL×Food+Pred×Food	-197.5	5.6	0.06	0.03	16.4
SL+Pred+Food+SL×Pred+Pred×Food	-197.0	6.1	0.05	0.02	20.9
SL+Pred+Food+SL×Pred+SL×Food+Pred×Food	-196.3	6.7	0.03	0.02	29.2
SL+Pred+Food+SL×Pred+SL×Food+Pred×Food+SL×P×F	-194.3	8.8	0.01	0.01	82.6
SL	-181.1	22.0	0.00	0.00	>100
SL+Pred	-178.7	24.4	0.00	0.00	>100
Pred+Food	-75.1	128.0	0.00	0.00	>100
Food	-72.0	131.0	0.00	0.00	>100
Pred	-35.3	167.7	0.00	0.00	>100

Supplemental Table 8: Models for treatment effect combinations and fish length (log-transformed) on total tissue C:N (also log-transformed) at the end of the experiment. *SL* represents standard length, which was a log-transformed and a covariate throughout this table. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Fish length consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue C:N At Exp. Conclusion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
SL+Pred+Food+Pred:Food	-158.0	0.0	1.00	0.44	1.0
SL+Pred+Food+SL:Pred+Pred:Food	-156.5	1.5	0.46	0.21	2.2
SL+Pred+Food+SL:Food+Pred:Food	-155.3	2.7	0.26	0.12	3.8
SL+Pred+Food	-154.3	3.7	0.16	0.07	6.3
SL+Pred+Food+SL:Pred	-153.9	4.1	0.13	0.06	7.9
SL+Pred+Food+SL:Pred+SL:Food+Pred:Food	-153.7	4.3	0.11	0.05	8.7
SL+Pred+Food+SL:Pred+SL:Food+Pred:Food+SL:Pred:Food	-151.9	6.1	0.05	0.02	21.3
SL+Pred+Food+SL:Food	-151.8	6.3	0.04	0.02	22.8
SL+Pred+Food+SL:Pred+SL:Food	-151.2	6.8	0.03	0.01	29.9
Pred+Food	-146.2	11.8	0.00	0.00	>100
SL+Food	-143.4	14.6	0.00	0.00	>100
Food	-132.2	25.8	0.00	0.00	>100
SL+Pred	-129.9	28.2	0.00	0.00	>100
SL	-124.8	33.3	0.00	0.00	>100
Pred	-112.3	45.7	0.00	0.00	>100

Supplemental Table 9: Models for treatment effect combinations and food consumption on *length specific* tissue C content at the end of the experiment. *Cons* represents food consumption, which was a log-transformed and a covariate throughout this table. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects.. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Length-Specific Tissue C At Exp. Conclusion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Cons	-118.6	0.0	1.00	0.51	1.0
Cons+Pred	-116.4	2.2	0.34	0.17	3.0
Cons+Food	-116.3	2.3	0.32	0.16	3.2
Cons+Pred+Food	-113.8	4.8	0.09	0.05	10.8
Pred+Food	-113.8	4.8	0.09	0.05	10.8
Cons+Pred+Food+Cons×Food	-112.3	6.3	0.04	0.02	22.9
Cons+Pred+Food+Cons×Pred	-111.3	7.3	0.03	0.01	37.7
Cons+Pred+Food+Pred×Food	-111.2	7.4	0.03	0.01	39.7
Cons+Pred+Food+Cons×Food+Pred×Food	-109.7	8.9	0.01	0.01	86.6
Cons+Pred+Food+Cons×Pred+Cons×Food	-109.6	9.0	0.01	0.01	91.2
Cons+Pred+Food+Cons×Pred+Pred×Food	-108.6	10.0	0.01	0.00	>100
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food	-106.8	11.8	0.00	0.00	>100
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food+C×P×F	-103.8	14.8	0.00	0.00	>100
Food	-101.3	17.3	0.00	0.00	>100
Pred	-75.5	43.1	0.00	0.00	>100

Supplemental Table 10: Models for treatment effect combinations and food consumption on *length specific* tissue N content at the end of the experiment. *Cons* represents food consumption, which was a log-transformed. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Length-Indep. Tissue N At Exp. Conclusion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Cons+Pred+Cons×Pred	-213.8	0.0	1.00	0.23	1.0
Cons+Pred+Food+Cons×Pred	-213.2	0.6	0.74	0.17	1.4
Cons+Pred+Food+Cons×Food	-212.1	1.7	0.44	0.10	2.3
Cons+Pred+Food+Pred×Food	-212.1	1.7	0.44	0.10	2.3
Cons+Pred+Food+Cons×Pred+Cons×Food	-211.9	1.9	0.39	0.09	2.6
Cons+Pred	-211.7	2.0	0.36	0.08	2.8
Cons+Pred+Food+Cons×Food+Pred×Food	-211.1	2.7	0.26	0.06	3.9
Cons+Pred+Food+Cons×Pred+Pred×Food	-210.5	3.3	0.19	0.05	5.2
Cons+Pred+Food	-209.3	4.5	0.11	0.03	9.3
Pred+Food	-209.3	4.5	0.11	0.03	9.3
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food	-209.0	4.8	0.09	0.02	11.0
Cons	-208.9	4.9	0.09	0.02	11.7
Cons+Food	-207.6	6.2	0.04	0.01	22.4
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food+C×P×F	-206.1	7.6	0.02	0.01	45.6
Food	-203.1	10.7	0.00	0.00	>100
1	-181.1	32.7	0.00	0.00	>100

Supplemental Table 11: Models for treatment effect combinations and food consumption on *length specific* tissue C:N at the end of the experiment. *Cons* represents food consumption, which was a log-transformed and a covariate throughout this table. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects.. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Length-Specific Tissue C:N At Exp. Conclusion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Cons	-161.8	0.0	1.00	0.40	1.0
Cons+Pred	-160.5	1.3	0.52	0.21	1.9
Cons+Food	-159.6	2.3	0.32	0.13	3.1
Cons+Pred+Food	-158.0	3.8	0.15	0.06	6.7
Pred+Food	-158.0	3.8	0.15	0.06	6.7
Cons+Pred+Food+Pred×Food	-157.6	4.3	0.12	0.05	8.4
Cons+Pred+Food+Cons×Pred	-157.3	4.5	0.10	0.04	9.6
Cons+Pred+Food+Cons×Food	-155.6	6.2	0.04	0.02	22.3
Cons+Pred+Food+Cons×Food+Pred×Food	-154.9	6.9	0.03	0.01	31.4
Cons+Pred+Food+Cons×Pred+Pred×Food	-154.8	7.0	0.03	0.01	33.0
Cons+Pred+Food+Cons×Pred+Cons×Food	-154.6	7.2	0.03	0.01	36.9
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food	-152.1	9.7	0.01	0.00	>100
Cons+Pred+Food+Cons×Pred+Cons×Food+Pred×Food+C×P×F	-149.3	12.6	0.00	0.00	>100
Food	-143.4	18.4	0.00	0.00	>100
Pred	-129.9	32.0	0.00	0.00	>100

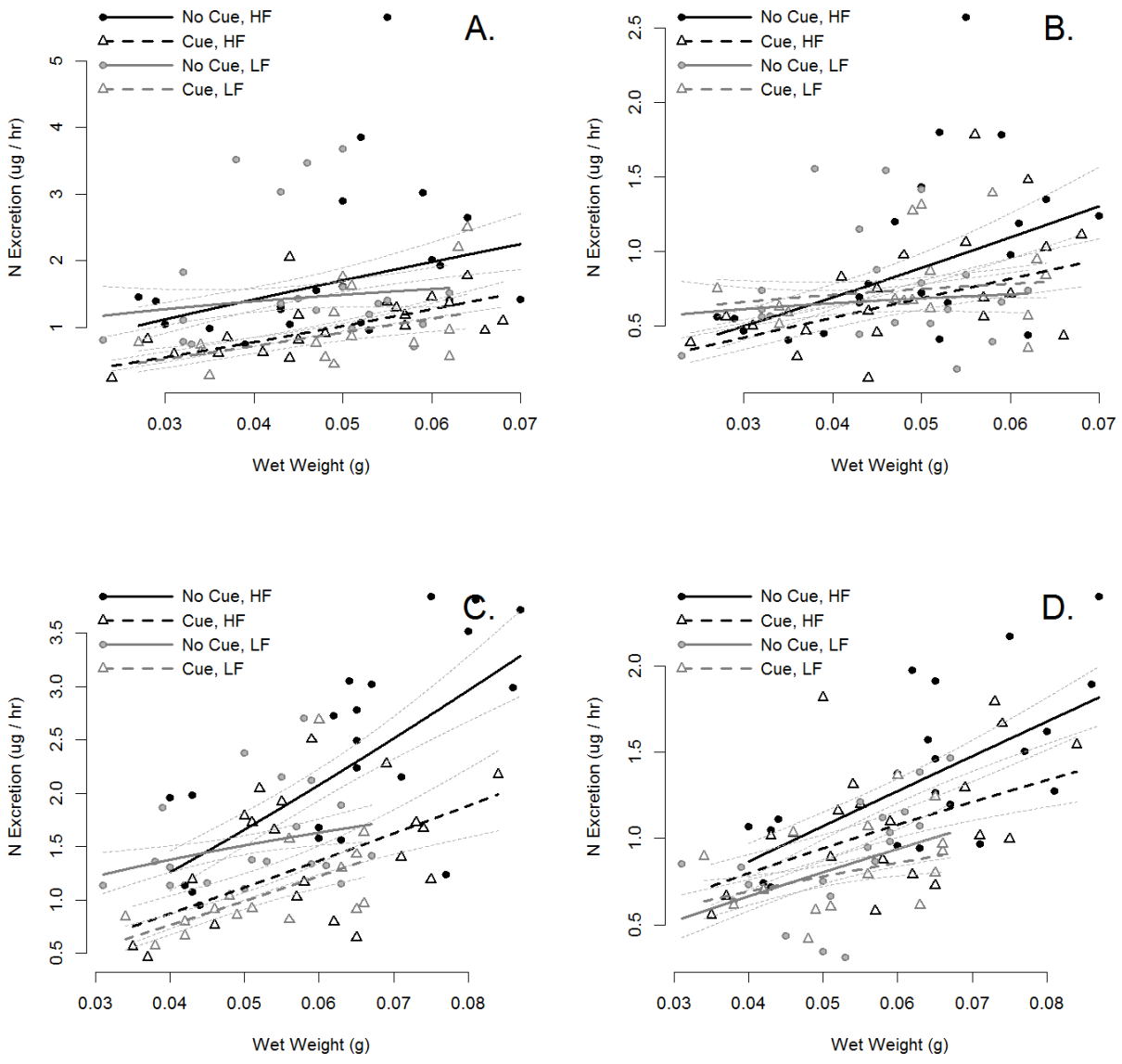
Supplemental Table 12: Models for treatment effect combinations and C consumption (*C cons*) on the estimated amount of C accreted as new tissue during the experiment. This is a measure of growth efficiency. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. C consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue C Accretion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
C cons	158.5	0.0	1.00	0.47	1.0
C cons+Food	160.4	1.9	0.38	0.18	2.6
C cons+Pred	160.8	2.3	0.31	0.15	3.2
C cons+Pred+Food	162.9	4.4	0.11	0.05	9.1
Pred+Food	162.9	4.4	0.11	0.05	9.1
C cons+Pred+Food+C cons×Food	165.4	7.0	0.03	0.01	32.4
C cons+Pred+Food+Pred×Food	165.5	7.0	0.03	0.01	33.1
C cons+Pred+Food+C cons×Pred	165.5	7.0	0.03	0.01	33.2
C cons+Pred+Food+C cons×Food+Pred×Food	168.0	9.6	0.01	0.00	>100
C cons+Pred+Food+C cons×Pred+C cons×Food	168.1	9.6	0.01	0.00	>100
C cons+Pred+Food+C cons×Pred+Pred×Food	168.1	9.6	0.01	0.00	>100
C cons+Pred+Food+C cons×Pred+C cons×Food+Pred×Food	170.8	12.3	0.00	0.00	>100
C cons+Pred+Food+C cons×Pred+C×F+P×F+C×P×F	171.9	13.4	0.00	0.00	>100
Food	210.5	52.0	0.00	0.00	>100
Pred	239.6	81.2	0.00	0.00	>100
1	245.1	86.7	0.00	0.00	>100

Supplemental Table 13: Models for treatment effect combinations and N consumption (*N cons*) on the estimated amount of N accreted as new tissue during the experiment. This is a measure of growth efficiency. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. N consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for Tissue N Accretion	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
N cons+Pred+Food+N cons×Pred	-125.9	0.0	1.00	0.35	1.0
N cons+Pred+Food+N cons×Pred+Pred×Food	-123.9	2.0	0.36	0.13	2.8
N cons+Pred+Food+N cons×Pred+N cons×Food	-123.3	2.6	0.28	0.10	3.6
N cons+Pred+Ncons×Pred	-122.8	3.1	0.21	0.07	4.7
N cons + Pred + Food × N cons	-122.8	3.1	0.21	0.07	4.7
N cons	-122.5	3.4	0.18	0.06	5.5
N cons+Pred+Food+Pred×Food	-122.0	3.9	0.14	0.05	7.1
N cons+Pred+Food+N cons×Pred+N×F+P×F+N ×P×F	-121.7	4.2	0.12	0.04	8.3
N cons+Pred	-121.5	4.4	0.11	0.04	9.2
N cons+Pred+Food+N cons×Pred+N cons×Food+P×F	-121.4	4.5	0.10	0.04	9.6
N cons+Pred+Food+N cons×Food	-121.2	4.7	0.09	0.03	10.7
N cons+Pred+Food	-120.5	5.4	0.07	0.02	15.1
Pred+Food	-120.5	5.4	0.07	0.02	15.1
N cons+Food	-120.1	5.8	0.06	0.02	18.1
N cons+Pred+Food+N cons×Food+Pred×Food	-120.0	5.9	0.05	0.02	19.5
Food	-72.8	53.1	0.00	0.00	>100
Pred	-33.8	92.1	0.00	0.00	>100

Supplemental Figure 1: N excretion by guppies at the outset (A and B) and conclusion (C and D) of the experiment when guppies were recently fed (A and C) or fasted for more than 6 hours (B and D). (A) At the outset of the experiment, guppies on either food ration (black vs. gray) excreted comparable amounts of N immediately after feeding, but excretion by both food levels was lower with predator cues than without (solid lines vs. dashed lines. (B) After a several hour fast, treatment effects on N excretion were minimal. (C) At the conclusion of the experiment, both low food level (gray vs. black) and predator cues (solid vs. dashed lines) reduced N excretion, but (D) the predator cue effect (solid vs. dashed lines) was much weaker after a 12 hour fast, especially on the low food treatment (gray lines). Thick lines represent best fit power regressions for each treatment group, with thin lines indicating the standard errors associated with these fits.



Supplemental Table 14: Models for treatment effects and fish wet weight on the estimated amount of N released as ammonia waste ***immediately after feeding*** on ***Day 3*** of the experiment. *Wt* represents the log-transformed wet weight of the fish at the time of measurement. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Weight and excretion were log-transformed to conform to the assumption of normal error distributions and allometric scaling laws. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to be the best model than the specified model.

Models for Fed N Excretion, Experiment Start	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Wt+Pred	85.7	0.0	1.00	0.32	1.00
Wt+Pred+Food+Wt×Pred	86.1	0.4	0.81	0.26	1.23
Wt+Pred+Food	87.6	1.9	0.38	0.12	2.61
Wt+Pred+Food+Wt×Pred+Wt×Food	88.2	2.5	0.29	0.09	3.45
Wt+Pred+Food+Wt×Pred+Pred×Food	88.7	3.0	0.22	0.07	4.45
Wt+Pred+Food+Wt×Food	89.6	3.8	0.15	0.05	6.80
Wt+Pred+Food+Pred×Food	90.1	4.4	0.11	0.04	8.90
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food	90.8	5.1	0.08	0.02	12.95
Wt+Pred+Food+Wt×Food+Pred×Food	92.1	6.4	0.04	0.01	24.01
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food+Wt×Pred×Food	93.7	8.0	0.02	0.01	54.11
Pred	94.3	8.6	0.01	0.00	>100
Food + Pred	96.0	10.3	0.01	0.00	>100
Wt	107.4	21.7	0.00	0.00	>100
Wt+Food	109.4	23.7	0.00	0.00	>100
1	114.2	28.4	0.00	0.00	>100

Supplemental Table 15: Models for treatment effects and fish wet weight on the estimated amount of N released as waste ***8 hours after feeding*** on ***Day 3*** of the experiment. *Wt* represents the log-transformed wet weight of the fish at the time of measurement. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Weight and excretion were log-transformed to conform to the assumption of normal error distributions and allometric scaling laws. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to be the best model than the specified model.

Models for Fasted N Excretion, Experiment Start	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Wt	72.6	0.0	1.00	0.26	1.00
Wt+Food	73.2	0.7	0.71	0.18	1.41
Wt+Pred+Food+Wt×Food	73.7	1.1	0.57	0.15	1.76
Wt+Pred+Food+Wt×Food+Pred×Food	74.6	2.0	0.36	0.09	2.75
Wt+Pred	75.0	2.4	0.30	0.08	3.36
Wt+Pred+Food	75.8	3.2	0.20	0.05	4.95
Wt+Pred+Food+Wt×Pred+Wt×Food	76.4	3.8	0.15	0.04	6.72
Wt+Pred+Food+Pred×Food	76.6	4.1	0.13	0.03	7.74
1	76.8	4.2	0.12	0.03	8.27
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food	77.4	4.8	0.09	0.02	11.00
Food	77.6	5.0	0.08	0.02	12.49
Wt+Pred+Food+Wt×Pred	78.3	5.8	0.06	0.01	18.08
Pred	79.1	6.5	0.04	0.01	26.35
Wt+Pred+Food+Wt×Pred+Pred×Food	79.3	6.8	0.03	0.01	29.54

Supplemental Table 16: Models for treatment effects and fish wet weight on the estimated amount of N released as waste ***immediately after feeding*** at the ***end of the experiment***. *Wt* represents the log-transformed wet weight of the fish at the time of measurement. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Weight and excretion were log-transformed to conform to the assumption of normal error distributions and allometric scaling laws. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to be the best model than the specified model.

Models for N Excretion, End of Experiment, Fed	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Wt+Pred+Food+Wt×Food	52.7	0.0	1.00	0.25	1.00
Wt+Pred	52.9	0.2	0.92	0.23	1.09
Wt+Pred+Food	53.4	0.7	0.71	0.18	1.41
Wt+Pred+Food+Wt×Pred+Wt×Food	54.4	1.7	0.43	0.11	2.34
Wt+Pred+Food+Wt×Pred	55.3	2.5	0.28	0.07	3.55
Wt+Pred+Food+Wt×Food+Pred×Food	55.4	2.7	0.26	0.07	3.78
Wt+Pred+Food+Pred×Food	56.0	3.3	0.19	0.05	5.17
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food	57.2	4.5	0.11	0.03	9.36
Wt+Pred+Food+Wt×Pred+Pred×Food	57.9	5.2	0.08	0.02	13.18
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food+Wt×Pred×Food	58.3	5.6	0.06	0.02	16.07
Wt	74.6	21.9	0.00	0.00	>100
Food + Pred	75.1	22.3	0.00	0.00	>100
Wt+Food	76.0	23.3	0.00	0.00	>100
Pred	82.8	30.1	0.00	0.00	>100
Food	98.7	46.0	0.00	0.00	>100

Supplemental Table 17: Models for treatment effects and fish wet weight on the estimated amount of N released as waste ***12 hours after feeding*** on ***Day 14*** of the experiment. *Wt* represents the log-transformed wet weight of the fish at the time of measurement. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. Weight and excretion were log-transformed to conform to the assumption of normal error distributions and allometric scaling laws. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to be the best model than the specified model.

Models for N Excretion, Day 14 Fasted	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Wt+Pred+Food	35.9	0.0	1.00	0.28	1.00
Wt+Pred+Food+Pred×Food	36.5	0.6	0.74	0.21	1.35
Wt+Pred+Food+Wt×Pred	37.4	1.5	0.48	0.13	2.10
Wt+Pred+Food+Wt×Food	38.0	2.1	0.35	0.10	2.86
Wt+Food	38.4	2.5	0.29	0.08	3.45
Wt+Pred+Food+Wt×Pred+Pred×Food	38.7	2.8	0.25	0.07	4.05
Wt+Pred+Food+Wt×Food+Pred×Food	38.9	2.9	0.23	0.06	4.34
Wt+Pred+Food+Wt×Pred+Wt×Food	39.7	3.7	0.16	0.04	6.44
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food	41.2	5.2	0.07	0.02	13.69
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food+Wt×Pred×Food	43.8	7.8	0.02	0.01	50.44
Wt+Pred	47.3	11.4	0.00	0.00	>100
Food + Pred	47.6	11.6	0.00	0.00	>100
Wt	47.9	12.0	0.00	0.00	>100
Food	53.0	17.0	0.00	0.00	>100
Pred	75.0	39.1	0.00	0.00	>100

Supplemental Table 18: Models for treatment effect combinations and N consumption per meal (*N cons*) on the estimated amount of N excreted per meal during the experiment, during the second week of the experiment. This measures how much of consumed N was released as ammonium within 12 h. *Pred* represents a fixed effect corresponding to the presence of predator chemical cues in the water (present or absent), and *Food* represents a fixed effect corresponding to the level of food ration fed to each guppy (high or low). Each model also contains "block" and "population" as random effects. N consumption was log-transformed to conform to the assumption of normal error distributions. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_I for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Models for N Excretion Per Meal	AICc	ΔAICc	Rel. lik.	w_i	w_i ratio
Wt+Pred+Food+Wt×Food	465.2	0.0	1.00	0.41	1.00
Wt+Pred+Food+Wt×Pred+Wt×Food	466.2	0.9	0.63	0.26	1.58
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food+Wt×Pred×Food	467.5	2.3	0.32	0.13	3.15
Wt+Pred+Food+Wt×Food+Pred×Food	467.5	2.3	0.32	0.13	3.16
Wt+Pred+Food+Wt×Pred+Wt×Food+Pred×Food	468.5	3.3	0.19	0.08	5.21
Wt+Pred+Food+Wt×Pred	475.1	9.8	0.01	0.00	>100
Wt+Pred+Food+Wt×Pred+Pred×Food	477.5	12.3	0.00	0.00	>100
Wt+Pred+Food+Pred×Food	479.8	14.5	0.00	0.00	>100
Wt+Pred	480.0	14.8	0.00	0.00	>100
Wt+Pred+Food	482.1	16.8	0.00	0.00	>100
Wt	488.4	23.1	0.00	0.00	>100
Wt+Food	490.3	25.0	0.00	0.00	>100
Food + Pred	490.7	25.5	0.00	0.00	>100
Food	511.4	46.2	0.00	0.00	>100
Pred	516.4	51.2	0.00	0.00	>100
1	528.1	62.9	0.00	0.00	>100

Supplemental Table 19: Table of effect size measures and associated standard errors for each of eight different response variables. Effect size and associated errors were calculated as unbiased Hedge's *d* (as in Nakagawa and Cuthill 2007). Excretion measures are size-corrected and log-transformed, as in Figure 3. Estimated effect sizes are reported ± their associated standard errors.

Response Variable	Low Food Effect Size	Predator Effect Size	Combined Effect Size
Food Eaten	-4.46 ± 0.65	-2.08 ± 0.44	-4.99 ± 0.72
Specific Growth	-1.91 ± 0.42	-0.71 ± 0.36	-2.13 ± 0.44
Tissue Energy Density	-1.62 ± 0.4	-0.95 ± 0.37	-1.7 ± 0.41
Tissue C:N	-1.66 ± 0.4	-1.13 ± 0.38	-1.8 ± 0.42
Excretion Fed, D1	-0.27 ± 0.34	-1.44 ± 0.39	-1.27 ± 0.38
Excretion Fasted, D1	-0.41 ± 0.35	-0.27 ± 0.35	-0.3 ± 0.35
Excretion Fed, D14	-0.26 ± 0.34	-1.17 ± 0.38	-1.63 ± 0.41
Excretion Fasted, D14	-0.97 ± 0.36	-0.88 ± 0.37	-1.38 ± 0.39

Supplemental Analysis 1: We fed a set of seven fishless tanks for the purpose of recovering uneaten foods, to estimate average leaching rates after 1 h. Each tank was fed 113.3 mg of ash free dry mass of food over two weeks. Recovery took place within 90 min after feeding. On average, we covered 56.9 mg of food from these tanks (± S.E. = 1.2 mg), for a recovery of 51%.

Supplemental Table 20: Number of blocks from each collection site in Trinidad. Guppies were introduced to the experiment as they were produced, resulting in uneven sample sizes related to the number of actively breeding adults at the time of the experiment. Arima LP is disproportionately represented in this dataset, but no population by treatment interactions were noted in our analysis.

Population	Number of Blocks
Arima "HP"	2
Arima "LP"	10
Aripo HP	2
Aripo LP	2

SUPPLEMENTAL MATERIALS – CHAPTER FIVE

Supplemental Table 1: Models for size-specific respiration rate of guppies reared with and without predation risk cues, measured in water both with and without predator cues. "Pred Rearing" (or just "Pred" in interaction terms) reflects whether the guppy was reared in water with predator cues. "Population" reflects the population from which the guppy is descended. "Cue" represents whether the incubation occurred in water with or without predation risk. Each model also contains "Fish ID" as a random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pop+Pred Rear+Cue+Pred Rear × Cue	-408.4	0.0	1.000	0.181	1.0
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue+Pred Rear×Cue+Pop×Pred Rear×Cue	-407.8	0.6	0.725	0.132	1.4
Pop+Cue	-407.7	0.8	0.685	0.124	1.5
Pop+Pred Rear+Cue+Pop×Pred Rear+Pred Rear×Cue	-407.4	1.0	0.597	0.108	1.7
Pop+Pred Rear+Cue+Pop×Cue+Pred Rear×Cue	-406.9	1.5	0.466	0.084	2.1
Pop	-406.9	1.5	0.461	0.084	2.2
Pop * Cue	-406.1	2.4	0.308	0.056	3.2
Pop+Pred Rear+Cue	-405.9	2.5	0.284	0.051	3.5
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue+Pred Rear×Cue	-405.7	2.7	0.261	0.047	3.8
Pop+Pred Rear	-405.2	3.3	0.195	0.035	5.1
Pop+Pred Rear+Cue+Pop×Pred Rear	-405.1	3.3	0.192	0.035	5.2
Pop * Pred Rear	-404.5	4.0	0.138	0.025	7.2
Pop+Pred Rear+Cue+Pop×Cue	-404.2	4.2	0.123	0.022	8.1
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue	-403.3	5.1	0.078	0.014	12.8
Pred Rear * Cue	-383.4	25.0	0.000	0.000	<100
Cue	-382.3	26.2	0.000	0.000	<100
No fixed effects	-381.1	27.4	0.000	0.000	<100
Pred Rear+Cue	-380.7	27.7	0.000	0.000	<100
Pred Rear	-379.5	28.9	0.000	0.000	<100

Supplemental Table 2: Models for size-specific excretion rate of guppies reared with and without predation risk cues, measured in water both with and without predator cues. "Pred Rearing" (or just "Pred" in interaction terms) reflects whether the guppy was reared in water with predator cues. "Population" reflects the population from which the guppy is descended. "Cue" represents whether the incubation occurred in water with or without predation risk. Each model also contains "Fish ID" as a random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pop+Cue	646.6	0.0	1.000	0.177	1.0
Pop+Pred Rear+Cue+Pop×Pred Rear	646.9	0.3	0.864	0.153	1.2
Pop+Pred Rear+Cue	647.2	0.6	0.746	0.132	1.3
Pop * Cue	647.2	0.6	0.743	0.131	1.3
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue	647.7	1.1	0.577	0.102	1.7
Pop+Pred Rear+Cue+Pop×Cue	647.9	1.3	0.531	0.094	1.9
Pop+Pred Rear+Cue+Pop×Pred Rear+Pred Rear×Cue	649.2	2.6	0.269	0.047	3.7
Pop+Pred Rear+Cue+Pred Rear × Cue	649.4	2.8	0.241	0.043	4.1
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue+Pred Rear×Cue	650.1	3.5	0.172	0.030	5.8
Pop+Pred Rear+Cue+Pop×Cue+Pred Rear×Cue	650.2	3.6	0.165	0.029	6.1
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue+Pred Rear×Cue+Pop×Pred Rear×Cue	650.5	3.9	0.141	0.025	7.1
Cue	651.0	4.4	0.110	0.019	9.1
Pred Rear+Cue	651.7	5.1	0.079	0.014	12.7
Pred Rear * Cue	653.9	7.3	0.026	0.005	37.8
Pop	679.3	32.7	0.000	0.000	>100
Pop * Pred Rear	679.6	33.0	0.000	0.000	>100
Pop+Pred Rear	679.8	33.2	0.000	0.000	>100
No fixed effects	683.2	36.6	0.000	0.000	>100
Pred Rear	683.8	37.2	0.000	0.000	>100

Supplemental Table 3: Models for respiration-specific excretion rate of guppies reared with and without predation risk cues, measured in water both with and without predator cues. “Pred Rearing” (or just “Pred” in interaction terms) reflects whether the guppy was reared in water with predator cues. “Population” reflects the population from which the guppy is descended. “Cue” represents whether the incubation occurred in water with or without predation risk. Each model also contains “Fish ID” as a random effects. The metric w_i corresponds to the relative likelihood of the specified model divided by the sum of relative likelihoods of all considered models, and it represents the probability that the specified model is the best model of the set of all considered models. The w_i ratio corresponds to the maximum w_i for the set of all considered models divided by the w_i of the specified model. This figure represents the number of times more likely the best model from a given set is to actually be the best model than the specified model.

Model Terms	AICcs	ΔAICc	Rel. Lik.	w_i	w_i ratio
Pop+Cue+Pop × Cue	568.2	0.0	1.000	0.464	1.0
Pop+Cue	570.1	1.9	0.384	0.178	2.6
Pop+Pred Rear+Cue+Pop×Cue	570.5	2.3	0.313	0.145	3.2
Pop + Pred Rear + Cue + Pop×Cue + Pred Rear×Cue	572.1	3.9	0.144	0.067	6.9
Pop + Pred Rear + Cue	572.3	4.2	0.125	0.058	8.0
Pop + Pred Rear + Cue + Pop×Pred Rear + Pop×Cue	573.8	5.7	0.059	0.027	16.9
Pop + Pred Rear + Cue + Pred Rear×Cue	573.9	5.7	0.058	0.027	17.4
Pop + Pred Rear + Cue + Pop×Pred Rear	575.5	7.3	0.026	0.012	38.7
Pop + Pred Rear + Cue + Pop×Pred Rear + Pop×Cue + Pred Rear×Cue	575.5	7.3	0.026	0.012	39.1
Pop + Pred Rear + Cue + Pop×Pred Rear + Pred Rear×Cue	577.2	9.0	0.011	0.005	89.4
Pop+Pred Rear+Cue+Pop×Pred Rear+Pop×Cue+Pred Rear×Cue+Pop×Pred Rear×Cue	577.7	9.5	0.009	0.004	>100
Pop	596.8	28.7	0.000	0.000	>100
Pop + Pred Rear	599.1	30.9	0.000	0.000	>100
Pop * Pred Rear	602.1	33.9	0.000	0.000	>100
Cue	614.5	46.4	0.000	0.000	>100
Pred Rear + Cue	616.7	48.5	0.000	0.000	>100
Pred Rear * Cue	618.1	49.9	0.000	0.000	>100
No fixed effects	639.9	71.7	0.000	0.000	>100
Pred Rear	642.0	73.8	0.000	0.000	>100